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THE USE OF PHOTOMICROGRAPHS IN TEACHING SPECIAL STAINS*

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Adequate instruction in histology to students of technology in the four weeks allotted to this subject, as recommended in "The Curriculum for Approved Schools", constitutes a problem of precise planning. If the most advantageous use of the available time is not a primary consideration in the teaching schedule, the students receive a little of this and that and, consequently, not much of anything.

Early in our training program, we recognized fully that the students had very little concept of the results of any given procedure. It became increasingly evident, therefore, that the problem might be resolved by the use of an aid frequently and successfully employed by teachers in many fields—the 35 mm colored slide, in this instance produced by photomicrography. Widely used, these slides might become a solution and hence a means to a comprehensively adequate teaching program.

Since it is virtually an impossibility to have the student fix, process, cut the tissues and finish the stains on all of the techniques taught in our laboratory in the available time, the slides on which the students work are prepared by the technologists. The student has ample time for practice cutting during time elapse periods in her staining techniques.

The photomicrographs were, therefore, prepared from slides of the same tissues given to the students for their techniques. An Ipscaphot Photomicrography Unit was used in the preparation of the slides, using Kodachrome F and Kodachrome A film.

The development of the teaching program tackles the problem of grappling with and mastering first things first. If the student never learns, by instruction and assiduous study, the difference between a well prepared section and one that is unsatisfactory, no aggregation of training methods in staining can possibly qualify her for a profession where skill and accuracy are keynotes to success. Photomicrographs were made from sections deficient in quality because of poor technique, of mediocre sections showing commonplace technical ability and of essentially good sections prepared by skillful artistry. In the manual compiled in our laboratory for student study, the difficulties encountered in sectioning and the means by which they are remedied are outlined in detail, and covered during a lecture period.

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The routine stain performed on all tissues is the next step demonstrated. Since the stain of choice by most pathologists for this purpose is a hematoxylin and eosin stain, photomicrographs were made from slides stained with Delafield[1] and Harris[2] hematoxylins counterstained with eosin. Despite the fact that this is a routine daily procedure, the causes for failure of proper staining must be fully demonstrated. A picture of what occurs when the amonia water or lithium carbonate is not completely removed after differentiation and before counterstaining gives conclusive evidence of the resultant stain defect, not easily forgotten. A photomicrograph of the cytoplasmic counterstain with and without acidification of the eosin demonstrates the importance of this step in the procedure. The photomicrographs show clearly the difference between good and poor hematoxylin and eosin stains.

The special stains employed for diagnostic purposes in any laboratory of pathology are essentially those chosen by the pathologist. It behooves the instructor of students, however, to consider the necessity of having the students not only informed of, but thoroughly familiar with, the methodology of many special stains, including new methods or modifications of established methods found in the current journals. Photomicrographs of stains using varied methods give the student an analogy and a comparative picture of what she will see in her own finished preparations.

The special stains used universally for many years in most laboratories must, of course, be a part of any teaching program covering good histologic techniques. The Verhoeff van Gieson stain for elastic tissue, by which the pathologist is helped in diagnosing blood vessel invasion of a malignant tumor, is an established technique used in our laboratory routinely for this purpose. This practical stain, however simple it may be in the hands of an experienced technologist, can give the students considerable difficulty if she does not realize the importance of a saturated solution of picric acid in making the van Gieson counterstain. Since the stain is used for a specific purpose, as previously stated, photomicrographs were made showing the blood vessel with deep blue-black elastic fibers and brilliant red collagen in a slide prepared precisely and skillfully and also in a slide where the van Gieson stain was not prepared with saturated picric acid. When the students are asked this question on a Board of Registry examination, I feel confident they can answer correctly and with less hesitation than if no visual aids were used. Elastic tissue is also demonstrated with Orcinol-New Fuchsin, a selective stain for elastic tissue[4] by which the elastic fibers are stained deep violet while the collagen remains unstained. An alternate method illustrated with photomicrographs is Gomori's Aldehyde-Fuchsin. A new stain for elastic tissue[5] by which the elastic fibers are stained deep purple to violet with a light green counterstain.

The collagen and reticulum of connective tissue have many more or less specific stains of considerable variety available for their demonstration. The Masson Trichrome[6] prepared from tissues fixed in Bouin's solution presents a picture with black nuclei, vermillion red cytoplasm and intense blue collagen. The simpler Gomori One-Step Trichrome[7] gives very satisfactory results after all fixatives except alcohol. The pic-

ture of this stain shows red muscle fibers, green collagen and blue to black nuclei. Here the student also sees how the red shades are weakened by the use of hydrochloric acid alcohol as a differentiator of the nuclear stain, and learns that the hematoxylin used in staining of the nuclei can be differentiated, if necessary, with picric acid in 50 per cent to 70 per cent alcohol followed by washing in running tap water.

Mallory's Aniline Blue Collagen Stain[8] and Heidenhain's Aniline Blue-Collagen Stain[9] are those chosen in our laboratory for teaching purposes. The intense blue collagen fibrils of Mallory's stain with the contrasting red fibrin are compared with Heidenhain's modification which shows deep blue collagen and reticulum, reddish orange muscle and blue mucin.

Since Bouin's solution is the routine fixative in our laboratory, the choice of a silver impregnation method for reticulum is the Laidlaw Technique[10] which presents a picture showing black reticulum on a pinkish red bockground. However, the student very likely may work in a laboratory where fixation is accomplished with the almost universally used 10 per cent formalin. We, therefore, teach the student Foot's Modification of Bielschowsky's Method for Reticulum[11] and Gridley's Reticulum Stain[12] on tissues fixed in formalin. The Foot Technique gives a picture of a dark violet to black reticulum on a pink background with pink nuclei. In the Gridley Technique the fine reticulum fibers are stained black on a light taupe background with red nuclei.

A modification of Mayer's Muci Carmine Stain[13] for use on Bouin's fixed tissues is our choice of stain for demonstrating mucin producing cells with mucin staining deep rose to red, nuclei black and other tissue elements yellow. The Alcian-Blue-Feulgen Stain of Mowry for Acid Mucopolysaccharides[14] presents a picture of blue acidic substance and magenta nuclei.

Bennhold's Congo Red Method[15] with pale pink to red amyloid and blue nuclei, and our modified methyl violet technique using Methyl Violet 5B[16] with purplish violet amyloid on a blue background, demonstrate amyloid staining metachromatically on paraffin sections. The van Gieson stain[17] while not specific for amyloid is an effective means of distinguishing this substance from collagen, with amyloid staining yellow and collagen red. In the first two methods, the student is acquainted with the necessity of an aqueous mounting medium which will preserve the stain rather than hasten its fading.

It is important for the student to realize how easily artifacts simulating fat may occur in fat stains on frozen sections. Our choice of method is Oil Red O in Propylene Glycol[18] which, in the hands of a skilled technologist produces a beautiful stain, with the fat staining bright red and the nuclei blue. The photomicrographs show the student the results of a good method poorly prepared and the same method skillfully prepared.

Gomori's Iron Reaction[19] is used to demonstrate bright blue iron pigment, red nuclei and pink to rose cytoplasm.

The importance of a well prepared Schiff reagent used in several techniques is demonstrated in a slide stained with the Mac-

Manus Periodic Acid Schiff Stain[20] where the hyalin deposits in glomeruli stain rose to purplish red in a good preparation.

The stains for bacteria and fungi are demonstrated to the student by several methods. The Grocott Stain for Fungi[21] using Gomori's Methenamine Silver Nitrate and the Gridley Stain for Fungi[22] in tissues as well as the Hotchkiss-MacManus Stain for Fungi[23] are photomicrographed to show clearly the difference between the methods. In the Grocott Technique the fungi are sharply delineated in black, mucin is taupe to gray, the inner parts of mycelia and hyphae are old rose and the background is green. The Gridley Technique shows deep blue mycelia, deep rose to purple conidia, deep blue mucin and elastic tissue on a yellow background. In the Hotchkiss-MacManus Technique the fungi stain red. The Brown-Brenn modified Gram Stain[24] with Gram positive bacteria blue, Gram negative bacteria and nuclei red and a yellow background is compared to Lillie's Gram Stain[25] with Gram positive bacteria blue black, Gram negative bacteria, nuclei and fibrin red and cytoplasm pink. A modified Ziehl Neelsen stain[26] shows acid fast bacteria bright red and nuclei blue. Fite's New Fuchsin Formaldehyde Stain[27] presents a picture of acid fast bacilli blue, nuclei brown, collagen red and smooth muscle yellow.

A preparation of the Wolbach Modification of the Giemsa Technique[28] on sections of bone marrow can be excellent or poor. The demonstration by photomicrography clearly gives the student a picture of good cell detail with blue nuclei and pink cytoplasm or a muddy picture with little or no cell detail. The Strumia Rapid Universal Blood Stain as presented by Powell in A Method for Embedding and Staining Bone Marrow Fragments[29] presents a picture of distinctly blue nuclei with a pinkish red cytoplasm.

The Gomori Burtner Methenamine Silver Method[30] for argentaffin cells is photomicrographed and used as a teaching aid. Here the student learns the importance of technical skill in determining impregnation time in the methenamine silver solution. At optimal impregnation time, the argentaffin cells are selectively well blackened. Too long impregnation in the silver solution blackens connective tissue, nuclei, smooth muscle and surface epithelium as well.

Myelin sheath is demonstrated by the method of Weil[31] giving a picture of grey-blue myelin sheath. An alternate technique is Klüver's Luxol Blue Stain[32] where there is a striking contrast of the Nissl-stained cells with that of the predominantly bluish or greenish blue fibers.

CONCLUSION

Much can be said and resaid about the effectiveness of a teaching program. No program of teaching students of histology in which the histologic techniques are not actually performed by the student can possibly teach her the reactions within a staining technique and the areas where errors can be made. The use of photomicrographs must, therefore, be a supplementary aid in student teaching and not a substitution for work of her own. The objectives of this type of experimental teaching cannot be evaluated properly until the program has been in use for several years. A summary evaluation, however, leads me to believe the

students are developing more interest in the field of histology and doing much better work. The lack of concern heretofore has not been inadequacy of materials but a poor approach in the presentation of an extremely important and interesting phase of technology.

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IN MEMORIAM

Sr. M. Leonette Wahl, Wisconsin . . . May 1958

Leonard R. Sanderson, Georgia . . . . . May 1958

James K. Bozeman, Ph.D., Florida. . . . July 1958

Death, to a good man, is but passing through a dark entry, out of one little dusky room of his father's house, into another that is fair and large, lightsome and glorious, and divinely entertaining.—Clarke

SEROLOGICAL DIAGNOSIS OF ACQUIRED HEMOLYTIC ANEMIA*

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In recent years many investigators have devised a variety of serological tests which have proved useful in better understanding the mechanism of red cell hemolysis. This report will discuss the role of laboratory procedures in confirming the clinical diagnosis of acquired hemolytic anemia and stress the number of cases in which these tests are of little value.

Ehrlich used the term "horror autotoxicus" to express his conviction that the body does not form antibodies against antigens present in its own tissues. The general truth of this principle has long been accepted. Normally, it seems that animals become tolerant of most of their body constituents in the course of development, the process starting in utero.[1]

Nevertheless it is now believed that in certain diseases antibodies are formed against the subject's own tissue antigens. The fundamental feature of autoimmune hemolytic anemia is a loss of this normal early acquired tolerance of one's own red cell antigens. The cause of the red cell destruction in many cases appears to be an antibody present in the patient's serum, capable of reacting with its own cells.

The first and clearest example of this occurrence was provided by Donath and Landsteiner's discovery of a hemolysin in the serum of occasional patients with syphilis and very rarely in individuals without this disease.[2] This antibody unites with red cells at low temperatures only, and causes hemolysis at body temperature in the presence of complement. A person so affected suffers intravascular hemolysis after the exposure of even a portion of his body to cold; the disease is called paroxysmal hemoglobinuria. There is no question here of the development of a destructive antibody. The antibody is unrelated to that which is responsible for the Wasserman reaction.

Autoantibodies against red cells are also produced in a variety of other circumstances. These are more restricted in their activity; they agglutinate but do not lyse the cells, and their agglutinative activity occurs only in the cold. Such antibodies are found in many patients with atypical pneumonia,[3] but they occur less regularly also in other diseases including acute bacterial infections, trypanosomiasis, Raynaud's disease, cirrhosis of the liver and lupus erythematosus. In fact many years ago Landsteiner[4] showed that such antibodies in low concentrations can be detected in normal plasmas at temperatures below 5°C.

Although it seems improbable that the cold hemagglutinins ordinarily cause damage at body temperature, the possibility that this may happen is suggested by Stats[5] finding that slight agitation of agglutinated cells suffices to break them down, if antibodies are present in high concentration.

Instances of acute hemolytic anemia have been seen to accompany atypical pneumonia[6] with high tiers of cold agglutinins. It has also been

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suggested that the physiologic jaundice of newborn infants may be caused by cold agglutinins, in these cases transmited from the mother.[7]

It was believed for some time that the manifest destruction of erythrocytes in acquired hemolytic anemia might be caused by antibodies absorbed to the cells, hence not demonstrable by serologic tests.[8] With the introduction by Coombs of the antiglobulin test for demonstrating antibody attached to cells this hypothesis became amenable to proof which was supplied in 1946.[9] These antibodies act at body temperature and can be eluted at 56°C. A degree of mystery still surrounds the question of mechanism of hemolysis for this is unlike the rapid lytic effect seen in the usual antibody-complement system.

Certain tissue enzymes such as Beta-glucuronidase, hyaluronidase, lysozyme, and others not yet studied may play a role in the mechanism of red cell destruction. Erythrocytes sensitized by specific antibodies are lysed in the presence of Beta-glucuronidase in physiologic concentrations.[10] Normal cells not coated with antibodies do not hemolyse under the conditions of this test.

These various serological observations formed the basis for a panel of tests that was used to study patients who presented clinical and/or hematological evidence of anemia due to an acquired hemolytic process.

Methods

The patient's erythrocytes are tested for the presence of globulin coating by means of the direct Coombs or antihuman globulin tests. This consists of adding the antihuman globulin rabbit serum to a two percent saline suspension of washed red cells. If a globulin coating is present hemagglutination takes place.

The patient's serum is tested for the presence of circulating antibodies by using the indirect anti-globulin test. Two volumes of the serum to be studied are incubated with one volume of a two per cent red cell suspension or panel of cells for one hour at 37°C. The cells are then washed and a direct Coombs test is performed on them. If antibodies are present in the serum capable of attaching themselves to antigens on the red cell, the cells become coated with globulin. This then reacts with the antihuman globulin rabbit serum and produces agglutination of the cells.

If the indirect Coombs test is positive, serial dilutions of the patient's serum are made to determine the titer of antibodies. The serum is studied further to see whether these antibodies will agglutinate saline and albumin suspended or papain treated red cells.

Elution of the antibody from the red cell is accomplished by heating the cells at 56°C. and recovering the supernatant materials for study.

Curtain paper electrophoresis is used in some cases to separate the antibodies into different globulin fractions. The gamma, alpha, and beta globulins are sometimes used to neutralize the antihuman globulin serum[2] in order to characterize the type of antibody coating the patient's cells.

Serial dilutions of patient's serum are tested against cells suspended in saline and albumin at 5°C. to determine the presence of cold agglutinins and hemolysins.

The Donath Landsteiner test[11] is used to study sera of patients with paroxysmal cold hemoglobinuria.

Serums to which concentrations of Beta-glucuronidase equivalent to 200 Fishman units were added are incubated at 37°C. for 24 hours with a 50 per cent suspension of red cells. Controls are made up of normal AB serums tested in the same way. The amount of hemoglobin in the supernatant is measured after twenty-four hour incubation.

Results

Three hundred cases of anemia suggesting a hemolytic mechanism were studied with this panel of tests.

In 31 cases, or approximately 10 per cent, there was a positive direct antiglobulin test. Twenty-seven cases, or 9 per cent of the serums contained circulating antibodies and gave a positive indirect antiglobulin test.

The titer of antibodies coating the red cells ranged from 2 to 2048. The majority of cases had a titer of 32. The range of titer of the indirect Coombs or circulating antibodies was 1 to 64.

Further data relating to these cases is summarized in Table I.

Table I

TYPE OF CASES GIVING A POSITIVE DIRECT ANTIGLOBULIN TEST

Admitting Diagnosis	Number of Positive Cases
Chronic lymphatic leukemia	6
Lupus erythematosus	4
Liver disease	3
Anemia of pregnancy	2
Bacterial or viral infections	5
Carcinoma	3
Diabetes	1
Congenital spherocytic anemia	2
Hemoglobin C disease	1
Paroxysmal hemoglobinuria	1
Anemia etiology unknown	3
Total	31

The red blood cell counts of these patients ranged from 1.5 million to 3.0 million cells per cubic mm. The reticulocyte counts range from 5 to 45 per cent.

The sensitized red cells from most, but not all, patients with acquired hemolytic anemia behave in a way similar to cells sensitized with incomplete anti-D. Indeed in some cases the antibodies attached to the patients' cells have been identified in whole or part as Rh antibodies.

Two patients with the genotypes CDe/Ce and cDE/cE had antibody coating on their cells that acted as anti-e and anti-c respectively. Antibodies eluted from the patient's red cells reacted with cells containing the e and c antigens in the respective cases and failed to sensitize cells that lacked the c and e factors. In such cases, transfusion with donor cells containing e and c would not be recommended since such blood would have a shortened cell survival time.

The case with the anti-e specificity was a symptomatic hemolytic anemia associated with chronic lymphatic leukemia. The case with the anti-c specificity was a case of chronic bleeding associated with toxic hepatitis. In both these cases the anemia ranged from 1.48 million to 2.5 million red cells per cubic mm., the reticulocyte count varied from 14 per cent to 25 per cent, the marrow showed accelerated erythropoesis compatible with a hemolytic process.

It must be pointed out that all the other globulin coatings eluted from

the other 29 patients' red cells failed to show any specificity for the known erythrocyte isoantigens; they appear to be directed against a species-specific and probably cell-specific antigen.

Given sensitized cells it is possible, by adding relatively pure globulin preparations to antihuman serum to discriminate between the different globulin fractions attached to the cell. Most of the patients, 27 cases, with acquired hemolytic anemia whose cells gave a positive direct Coombs test due to antibodies of the warm (37°C.) type seem to have erythrocytes coated with the gamma globulin fraction. Antihuman globulin serum neutralized with pure gamma globulin failed to agglutinate the cells from these 27 cases. Also fractions of the eluted antibody was found in the gamma globulin fraction obtained by curtain electrophoresis.

In the other four cases the material absorbed to the patients' red cells has been shown to be composed mainly of beta and alpha$_2$ globulins; the sera of these patients giving this type of reaction contained nonspecific cold agglutinins in high concentrations.

Rabbit antiglobulin (Coombs) sera contain a variable amount of anti-gamma, anti-alpha$_2$ and anti-beta globulin, and this influences their reactions with different types of sensitized red cells. The optimal dilution of the serum depends on the concentration of these antiglobulins and is usually different for gamma and non-gamma antibodies respectively. In using the antiglobulin test for the detection of antibodies, it is unlikely that a single fixed dilution of the serum will satisfactorily agglutinate all types of sensitized red cells. Three of the cases would have been reported as negative had not a rabbit serum with a high titer of anti-alpha$_2$ and anti-beta globulin been used.

Many samples of human sera are capable of agglutinating cells at O°C. The normal cold agglutinin titer is found to be not greater than 16 or 32. There is no evidence that normal cold agglutinins exhibit any blood group specificity.

The four cases of high cold agglutinins with positive antiglobulin tests behaved similar to the incomplete cold agglutinin reported by Dacie.[12] Red cells incubated with these serums for two hours at 0—4°C. gave a positive reaction with antiglobulin serum. This incomplete cold antibody binds firmly on to red cells only in the presence of factors present in fresh serum; the optimum pH for binding is 8.0. Non-gamma globulin antibodies sensitize red cells to an antiglobulin serum only in the presence of complement. Thus the presence of such antibodies may be overlooked, if plasma, rather than fresh serum is used. Serum which has been heated at 56°C. does not sensitize red cells. Heparin, oxalate or citrate added to serum also inhibit the sensitization.

In this series the four cases with a positive direct Coombs test and negative indirect test at 37°C. had cold agglutinin titers ranging from 128 to 5000. These represented one case each of chronic lymphatic leukemia, hepatitis, lymphosarcoma and congenital spherocytic anemia.

Of the 27 cases with a positive direct anti-globulin test and autoagglutinins at 37°C. (indirect antiglobulin), only seven had elevated cold agglutinin titers. The remaining twenty cases had cold agglutinins within normal range.

In the 269 cases studied with negative antiglobulin tests there were 18 cases with high titers of cold agglutinins ranging from 256 to 10,000. Additional data is summarized in Table II.

Neutralization of these serums with different fractions of human globulin indicated that the majority of cold agglutinins are non-gamma globulin.

Table II

TYPE OF CASES ASSOCIATED WITH HIGH TITERS OF COLD AGGLUTININS

Admitting Diagnosis	Number of Cases	Range in Titer
Viral or bacterial infections	5	256—10,000
Anemia, etiology unknown	2	256— 5,000
Chronic lymphatic leukemia	2	128— 5,000
Liver disease	4	128— 5,000
Carcinoma	2	128— 4,000
Infectious mononucleosis	1	128— 2,000
Lupus erythematosus	2	256— 512
Total	18	

* Anemia ranged from 2.5 to 3.8 million red cells per cubic mm.
** Reticulocyte counts ranged from 4 per cent to 12 per cent.
*** The direct antiglobulin test was negative in all these cases.

The serum of patients with acquired hemolytic anemia of the cold type almost always give a positive indirect antiglobulin test with normal cells at 20°C. In many cases binding of incomplete cold antibody also occurs at 37°C., though less strongly and only if the serum is first slightly acidified.[13]

Sera which give a positive Donath Landsteiner reaction usually have only a normal titer of cold agglutinins. Only one of the two cases of cold paroxysmal hemoglobinuria in this series had a positive Coombs test and a high titer of cold agglutinins.

Paroxysmal cold hemoglobinuria was at one time considered to be invariably associated with syphilis. However, according to Dacie[14] in some cases the serologic reactions for syphilis are positive during the attack but become negative when the patient recovers. Neither of the two cases in this series were associated with syphilis.

Crosby[15] reported that when defibrinated blood from certain patients with leukemia or disseminated neoplasms is incubated under oil for 24 hours at 37°C. the red cells undergo hemolysis, and 50 to 500 mg. of hemoglobin per 100 ml. of plasma may be released.

In four cases of chronic lymphatic leukemia, one case of acute monocytic leukemia, one case of acute lymphocytic leukemia, three cases of lupus erythematosus, two cases of lymphosarcomas, and four cases of disseminated neoplasm there was increased hemolysis of the patients' cells after 24 hours at 37°C. under oil. The Coombs test was negative before and after incubation.

All 300 cases were studied for the effect on red cell hemolysis of a normal non-lytic concentration (200 Fishman units) of the enzyme Beta-glucuronidase incubated for 24 hours in the patient's serum. Twenty-nine of the thirty-one cases with positive direct antiglobulin tests showed increased hemolysis over the controls ranging from 9 to 26 per cent liberated hemoglobin. Incomplete cold agglutinins which did

not normally sensitize red cells, in the presence of Beta-glucuronidase coated the cells so that the direct Coombs test became positive.

In 25 cases where there was no serological evidence of abnormal antibodies, there was increased hemolytic activity of the patient's serum in the presence of Beta-glucuronidase. The hemoglobin liberated after incubating normal red cells, patient's serum and the enzyme for 24 hours at 37°C. ranged from 6 to 19 per cent.

Results Continued

In 300 cases with clinical or hematologic evidence of hemolysis the laboratory could provide a confirming serological diagnosis in only 31 cases using standard procedures. Making use of newer concepts not yet firmly established the number of cases with suggestive findings was 58. Using all the hemolytic tests available to this department the total number of cases with positive findings was brought to 89 or 29 per cent of the cases studied.

The remaining 211 negative cases had as good evidence for hemolysis as measured by unexplained anemia, reticulocytosis and accelerated marrow erythropoesis. These serologically negative patients had a wide range of diseases such as lymphomas, lupus erythematosus, liver disease, carcinomas, bacterial and virus infections, pernicious anemia, congenital spherocytic anemia and a number with the cause of the anemia not yet determined.

Discussion

From this large series of cases it is obvious that there is much about red cell destruction that is still a matter of speculation and many features need clarification e.g., the nature of the globulins coating the red cells and giving positive antiglobulin tests; the relationship between cases with positive antiglobulin tests and those in which the test is negative, the site of the "autoantibodies," and most important and obscure of all, the reason for their formation.

The findings of this study are consistent with the hypothesis that the globulin absorbed by the patient's cells is in some cases antibody globulin. There is the possibility that gamma globulin is bound to the red cells through the intermediary of an adsorbed bacterial or viral product or chemical; i.e., the globulin is an antibody directed against the adsorbed material rather than directed against the red cells.[16]

Against this hypothesis is the finding that the globulin may have a particular Rh specificity and that it is possible in a few cases to elute globulin from the patient's red cells and to demonstrate that the eluted globulin will attach itself to perfectly normal corpuscles. However, it is impossible to be certain that an intermediary between cell and globulin may not operate in some cases. Indeed this possible mechanism has the added attraction that it is unnecessary to postulate that the body has overcome its normal reluctance to devevlop antibodies against its tissues.

It would seem that the present serologic tests for detecting red cell agglutinins and hemolysins are not sensitive enough in the majority of cases. That is, if these are the only important factors responsible for hemolysis. There is a great deal of evidence in the literature and in

every day practice to support the theory that other agents may contribute to destructive hemolysis of red cells.

Investigators on cold hemoglobinuria and on paroxysmal nocturnal hemoglobinuria have drawn the attention of hematologists towards serum complement.[17] Properdin has been mentioned in connection with hemolysis in P.N.H. and bacteriolysis. Various tissue enzymes have been implicated as possible lytic mechanisms. These and many other factors should be investigated as to their true role in acquired hemolytic anemia.

Measurements of red cell survival have revealed that there are many conditions, without serological evidence of antibodies, in which survival is diminished by a random destructive process. Red cells "coated" with antibody are not necessarily destroyed at an increased rate. Thus Loutit and Mollison[18] found that red cells from patients with acquired hemolytic anemia survived normally when transfused to normal recipients even though the red cells gave a positive direct antiglobulin test for weeks after transfusion. In acquired hemolytic anemia in remission the direct antiglobulin test may be strongly positive although there are no signs of red cell destruction. Thus it must be concluded that mere coating of the red cells with antibody does not itself interfere with their survival.

Since the true mechanism of red cell destruction is merely speculation, the present laboratory tests can do no more than divide the cases of acquired hemolytic anemia into three groups. (1) Those of the "autoimmune" type which give positive results with the standard techniques, such as the antihuman globulin test, agglutinins and hemolysins at 37°C., and cold agglutinins (Incomplete) at 5°C. (2) Those patients whose serum gives evidence of hemolytic activity only upon prolonged incubation at 37°C. (3) The many cases which are considered clinically and hematologically to be hemolysing their red cells at an increased rate, but who consistently give negative results when tested for the presence of lytic agents.

Summary

Three hundred patients presenting symptoms of acquired hemolytic anemia were studied serologically with a panel of tests.

Thirty-one cases or 10 per cent gave a positive direct antiglobulin test. The globulin coating in two cases showed an anti-e and anti-c specificity. Twenty-two cases, or seven per cent, had elevated titers of incomplete cold agglutinins. In fifteen cases there was increased hemolysis of red cells incubated under oil for 24 hours at 37°C. In fifty-four cases there was evidence of hemolysis in a system incubated with Beta-glucuronidase.

There were only eighty-nine cases or twenty-nine per cent, that gave positive results when the entire panel of tests was used. 211 cases, or 71 per cent, failed to give positive results with any of these serological procedures.

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CLINICAL APPLICATION OF RADIOACTIVE PHOSPHORUS AND GOLD*

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The introduction of artificially produced radioactive isotopes in clinical medicine has given us a new approach to both the diagnosis and the treatment of disease. As medical technologists, your field of interest is chiefly in the use of radioisotopes in various clinical and laboratory tests as an aid in diagnosis. Some of you have had the opportunity to utilize radioisotopes as a research tool or in clinical laboratory techniques. As these tests are improved there will be more wide spread use in the laboratory and therefore a greater need for medical technicians who are adequately trained. Unfortunately there are too few institutions offering a training program for medical technologists in this new field. Basically, the isotope technical procedures are in many ways similar to those that you perform every day except that one must acquire the technique of a new laboratory test plus the proper method of measuring and handling radioactive material in order to minimize the radiation hazard to the patient; to the laboratory and to the lab personnel. One should also be familiar with the therapeutic uses of some of the isotopes since the radiation effect in the patient will alter certain laboratory findings such as the hematocrit, the white blood count, the red cell count and so forth.

Artificial radioisotopes are used in clinical medicine in three ways: First, as a tool for research; second, as an aid in diagnosis; and third, as a method of administering ionizing radiation for the treatment of disease. The two isotopes which I am to discuss—radiophosphorus and radiogold are among the most commonly used in clinical medicine today. Both have proven extremely valuable, especially in the field of radiation therapy. Radioactive phosphorus or P 32 is made in the nuclear reactor; it emits only beta radiation; has a half life of 14.3 days and decays to stable sulfur. All tissues of the body contain phosphorus and when radiophosphorus is introduced into the body it will be available to all tissues except that it will concentrate more rapidly and to a higher degree in the bone marrow, lymph nodes, liver, spleen and the brain tissue. In rats 98 days after the administration of P 32, 92 percent of the retained phosphorus is present in the skeleton.

The nuclear portion of the cell contains the highest concentration of the phosphorus. The need of phosphorus is greater when the mitosis proceeds at a relatively rapid rate. Consequently P 32 tends to concentrate three to five times more heavily in cancer cells than in non-cancerous tissue. Therefore, P 32 has been made use of as a diagnostic aid in malignant tumors of the breast, of the eye, and for brain tumor localization. The technical procedures in each of these three tests are basically the same. It involves the oral or the intravenous administration of a tracer dose of P 32 (about 300 to 500 microcuries of P 32) and then the measurement of the concentration of the radiophosphorus in the suspected area immediately or 24, 48, or 72 hours later. The measurements

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are made using a Geiger-Mueller type counting tube most sensitive to the beta rays of P 32. Comparable measurements are made over the various segments of the normal eye or breast to obtain the percentage of differential concentration. The localization of brain tumors requires the surgical removal of a bone flap and then the use of a specially designed Geiger tube to make the measurements and locate the tumor and its limits. The radioactivity of tumor tissue in the brain is from five to one hundred times greater than the adjacent normal tissue. In recent years many clinics are using human serum albumin tagged with radioiodine as an indicator for brain tumor localization. This isotope has the advantage over radiophosphorus as it can be detected and measured on the external surface of the skull without the need for surgical exploration.

Radiophosphorus is also used to determine blood volume. The clinical importance of accurately determining blood volume has become well recognized. The Evans Blue Dye Test has been used but has many disadvantages. The following are the advantages of the radioactive technique for measuring blood volume: 1. The amount of radioactivity employed is extremely small and is no hazard to the patient or to the technician. 2. The blood volume can be repeated as often as the need requires. 3. The procedure is entirely independent of color or the presence of opaque material and is independent of hemolysis.

When the patient's red blood cells are incubated with a solution of sodium acid phosphate labeled with P 32, the radiophosphorus enters the red blood cells. Most of the red blood cell content is in the form of easily exchangeable organic acid soluble phosphorus. P 32 remains in the red blood cells long enough so that the labeled red cells can be injected into the patient for blood volume studies using the principle of isotope dilution. The total red cell volume is first calculated and then the total blood volume using standard formula. Newer methods are employing I^{131} tagged human serum albumin and either the plasma volume or the total blood volume may be determined directly. So accurate is this method that it has been used for the determination of hematocrit values.

The first artifically produced isotope to be used in the treatment of human disease was radiophosphorus. In 1939, Dr. John H. Lawrence of the University of California treated several cases of chronic leukemia and in the following year initiated the use of the isotope for the treatment of polycythemia vera. The use of P 32 as a method of administering ionizing radiation to tissues has opened up an entirely new field in radiation therapy. You are familiar with the conventional method of treating malignant tumors by using x-rays applied externally or the use of radium sources for intracavity tumors such as those of the cervix and uterus. By administering radiophosphorus orally or intravenously we are giving the patient internal irradiation wherever the radioactive material is trapped by its metabolic processes. In other words, it is somewhat like placing a large number of x-ray machines inside the patient's body.

It is well known that ionizing radiation inhibits cell division in the early prophase of mitosis. P 32 is more effective than x-rays in inhibiting the proliferation of white and red blood cells because P 32 is deposited

in the bone marrow at the source of this proliferation. It gives off radiation constantly which tends to irradiate each cell thus inhibiting the proliferation of each cell as it reaches the particular time of prophase. P 32 is also concentrated in rapidly dividing cells elsewhere in the reticuloendothelial system. Because of the localization of P 32 it is made use of in the treatment of polycythemia vera and selective cases of chronic leukemia. Radiophosphorus is one of our most effective methods of treatment of polycythemia vera.

A correct diagnosis is essential before starting treatments. This requires complete clinical and laboratory examination of the patient including the determination of total blood volume, the plasma, and red cell volume, the hematocrit, bone marrow studies, and so forth.

Once the diagnosis is established the following plan of treatment is used in our clinic:

If the patient's symptoms are severe and the hematocrit is extremely high a phlebotomy of 500 cc. of blood is done and may be repeated before P 32 treatment is started. The dose of P 32 is 50 microcuries per kilo of body weight and the average is 2 to 5 millicuries given intravenously in the form of soluble sodium acid phosphate. The patient is seen every three to four weeks at which time a complete blood study is done. The treatment is continued until the clinical symptoms are relieved and the blood picture returns to normal. There is no radiation sickness induced by this method of treatment. The average number of doses is about 5 and the average remission will last for approximately three years and then the patient will require another course of treatment. The only complication is the over depression of the bone marrow which may produce a leukopenia or a severe anemia.

Radiophosphorus is less effective in the treatment of leukemia than in the treatment of polycythemia. It is of no value in the treatment of acute or sub acute leukemia. P 32 appears to produce better results in the chronic granulocytis leukemia than in the chronic lymphocytic leukemia. We do not use P 32 in the chronic leukemias if there is evidence that the disease is in an acute phase. The technique of treatment and management is similar to that used for polycythemia. The average duration of life for P 32 treated cases of chronic granulocytic leukemia is about three and one half years. This is comparable to results obtained by other methods of treatment. The greatest advantage of the treatment with P 32 is its ease of administration and the complete lack of radiation sickness.

Radioactive chromic phosphate is another form of P 32 used in the treatment of cancer. A sterile colloidal suspension of insoluble chromium phosphate is injected into either the pleural or peritoneal cavities or both for the control of fluid production resulting from metastatic cancer. Twenty-four hours after the injection of P 32 most of the radioactive material will be found deposited on the serous surfaces of the cavity in which it has been introduced. The purpose of the colloidal suspension is to prevent the P 32 from being absorbed into the general circulation through the serous membrane. This technique of treatment is an example of the mechanical placement of a radioactive substance in the body so that it can have intimate contact directly with cancer cells. It has been

shown experimentally that ionizing radiation given in this fashion can destroy cancer cells. The clinical results indicate that this is a worth while procedure because when effective it eliminates the need for frequent removal of fluid thereby preserving the patient's strength and keeping the patient more comfortable.

The second radioactive isotope under discussion is radioactive gold or AU^{198}. It is produced in the reactor; it emits beta and gamma rays; it has a half life of 2.7 days and decays into stable mercury.

In 1954 we were the first to demonstrate the passage of fluid from the chest to the abdomen and vice versa by using radiogold as the indicator. The patient under investigation had fluid in one of the chest cavities and in the peritoneal or abdominal cavity. The fluid was due to the spread of cancer cells to these two cavities. Radiogold was injected into the chest cavity containing the fluid and by frequent tapping of the fluid from the abdomen through a polyethylene tube we could recover a certain percentage of the radiogold previously injected in to the chest. At a later date the radiogold was injected into the abdominal cavity and by frequent removal of the fluid from the chest we demonstrated the movement of fluid from the abdomen to the chest by the increasing concentration of the radiogold in the fluid removed from the chest. The exact dynamics of this transport of radioactive material is not yet proven. This is a demonstration of the use of radiogold as a method of clinical investigation.

The chief clinical uses of radioactive colloidal gold are: 1. For the control and prevention of cancerous fluid formation in the chest and abdomen; a use similar to that of radioactive chromate phosphate. 2. By injection directly into the prostate for the treatment of selective cases of prostatic cancer. 3. By direct injection into the cervix for cancer of this structure. 4. Given intravenously into the blood stream for the treatment of chronic leukemias. When given intravenously radiogold concentrates chiefly in the reticuloendothelial system, namely the liver, spleen and lymph nodes. Because of this localization, attempts are being made to use it to treat other conditions such as: Hodgkins disease, lymphomas, and so forth.

Our experience with radiogold has been limited to the treatment of cancerous fluid collections in the chest and abdomen. The laboratory plays an important part in this work because of the need for excellent technique for detecting cancer cells in the fluid taken from the serous cavity or finding the cancer cells in washings or direct smears from these cavities. The early detection of the presence of cancer cells before the fluid formation begins and the treatment of the patient at this time will be an important step in the control of certain types of cancer using radioactive material. We have applied this principle in using radioactive gold for the treatment of ovarian cancer where we can demonstrate that the peritoneum has been contaminated by cancer cells. Our results have been extremely encouraging since we began this program in 1952.

Summary

Radiophosphorus and radiogold are being used daily in the practice of clinical medicine. Both illustrate how isotopes can be utilized in research, in diagnosis and in treatment of disease. As our knowledge of the application of these isotopes and other radioactive substances increases they will become a part of every medical laboratory. The medical technologists will be using radioactive isotopes and radiation counters as frequently as they use test tubes and microscopes today.

GASTRIC CYTOLOGY—A PROMISING SPECIALIZATION*†

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Exfoliative cytology in the field of medical investigation and recently in the work of hospital laboratories has brought to the medical technologist a new challenge and a new area for exploration and study with opportunity for specialization.

Although experiment may in time prove that in ribonucleic acid, desoxyribonucleic acid, in the chromosomes, in the mitochondria, or in some as yet undiscovered factor, lies the explanation for malignancy, our best means of recognizing cancer today is by cell morphology.

After painstaking investigation by pioneers in the field and with concentrated effort since 1941 by Papanicoloau and others, vaginal and cervical cytology are now accepted as valuable diagnostic tools. Techniques and interpretation are well standardized. However, the diagnosis of gastric cancer remains a challenging problem. The mortality rate of carcinoma of the stomach in the United States is 25,000 to 40,000 annually and represents approximately 18 percent of the total deaths from malignancy.[2] To the early recognition of such cases, gastric cytotechnologists are directing their efforts.

Gastric cytology is, for the present, a specialized area for investigation and laboratory diagnosis.

Ideally, gastric cytology should be performed within a hospital group which serves in clinical and investigative gastroenterology. This assures close liasion between the clinician attending the patient and the gastric cytologist. The gastric aspirate and lavage for cytological examination should not be collected as a routine procedure of the hospital ward or out-patient department. Rather, it should be collected, under professional supervision, by a specially trained person. In one hospital where gastric lavages are collected routinely on the ward, almost 50 percent unsatisfactory smears were reported.[17]

Gastric cytology can be a valuable adjunct to other diagnostic means. A recent article by Seybolt and Papanicolaou shows figures which indicate the value of combined study.[12]

Cytological examination alone gave a correct diagnosis in 66.6 percent of the positive cases studied.

Radiological examination alone gave a correct diagnosis in 68.5 percent of the positive cases studied.

In combination the correct positive diagnosis increased to 88.7 percent.

Because gastric cytology is still in its adolescence, each new modification, whether it be in preparation, intubation, staining, or screening, should be noted. The alert cytotechnologist records all findings—studies, classifies, and subsequently relates them. From these records will ultimately come a more detailed classification of the variables found in cells from the gastrointestinal tract.

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It is the responsibility of the cytologist to perfect techniques and help standardize interpretation in order that he may recognize the malignant gastric cell in its earliest manifestation.

THE GASTRIC CYTOTECHNOLOGIST

Cytotechnology is in itself a specialized field. The new recruitment film, "The Human Cell and The Cytotechnologist," and the establishment of a new A.S.C.P. registry examination for cytology specialists points out current interest.

Gastric cytotechnologists should be individually trained for their unique job. The accuracy of results is directly proportional to the processing of gastric specimens by well-trained, interested personnel.[3] It is desirable that the person who reads the smears be the one who has collected the specimen. She has interest in the patient, in the specimen, in reading the slides, and in the final report.

The gastric cytotechnologist should have a good clinical background, a thorough knowledge of anatomy and physiology. She must be meticulous and must be manually dexterous. Her attitude should be one of enthusiasm, persistence, and intellectual curiosity coupled with sound judgment. She should be an observant microscopist. Her job involves patient-technologist relationship on a highly professional basis. Because gastric intubation is seldom received with enthusiasm on the part of the patient, technologist-patient rapport is most important. The personality and approach of the technologist often determine the success of gastric intubation, since it can be vividly demonstrated that the gastric mucosa reflects the emotional status of a patient.

METHODOLOGY

Avoid doing cytological examinations under any but optimum conditions.

Patient Preparation:

Proper patient preparation is essential. The stomach should be empty. (Schedule cytology at least 48 hours after upper G.I. series.) Retained food, debris, and barium are deterrents to a satisfactory lavage specimen. A suggested regimen is:

1. Encourage water intake day before examination.
2. Light supper on day preceding examination; if gastric retention is suspected, restrict to clear fluids only.
3. Nothing by mouth from midnight.

Intubation:

Techniques involving balloons, brushes, and special tubes with or without the use of a mucolytic factor have been tested by various investigators.[1,6,10] Levin tube lavage and Ewald tube lavage have been used successfully in this laboratory for more than two years. Levin tube intubation in the hands of an experienced person can be performed with minimum difficulties. Ewald tube lavage is used by physicians prior to gastroscopy examination.

It is beyond the scope of this paper to compare the numerous tech-

niques recommended for collecting gastric fluid. Although it is possible to specify one detailed method, each technologist must develop her own distinct techniques; timing becomes a matter of feeling; determination of a satisfactory lavage is a matter of judgment; and proper handling of the tube is individualistic. With experience comes the necessary discrimination. What follows is, therefore, an outline of what has been a satisfactory procedure with a few suggestions which may be helpful.

Procedure for Levin Tube Lavage:

With the patient in a sitting position, an iced #14 Levin Tube is introduced through the nostril. When the tube reaches the pharynx, the patient is instructed to swallow some water and the tube is pushed through gently but quickly. Slowing or stopping often causes gagging and retching. The patient should be instructed not to swallow saliva or mucus which may add to the confusion of interpretation.

Severe coughing or gasping as the tube passes the pharynx may indicate that the tube has entered the trachea. It should be pulled back to the nasopharynx and reintroduced. Some patients do not react even if the tube is in the trachea so its position should be checked. Several techniques have been employed to determine location. One cytologist holds the free end of the tube near the patient's hair. Air from the trachea causes the hair to move. Another checks by watching for bubbles when the free end of the tube is immersed in water. Still another uses the stethoscope to listen for the gurgle of injected air. Merely listening for breathing sounds may tell the location of the tube.

Gentle suction will usually recover gastric fluid as soon as the Levin tube is in the stomach. A 50 cc syringe (more comfortably handled than the 100 cc recommended by some workers) is attached and the stomach completely aspirated. If retention of food is encountered, the stomach may be washed with copious water or saline but any further procedure postponed until the next day.

It is advisable to do at least two lavages. The first will eliminate excess mucus, acid, debris, or regurgitated bile when present; the second (or third or fourth) will usually contain the best preserved gastric cells. For the final lavage, approximately 50 cc Ringer's solution or saline is instilled into the stomach, aspirated, and reinstilled vigorously several times in order to abrade the mucosa maximally.

The tube is moved up and down to recover all the residual secretion and to sample as much of the stomach as possible remembering that material is very apt to pocket itself in the stomach folds. Moving the patient from side to side or even placing him in Trendelenburg position on the bed may aid cell collection. Exfoliating cells can be caught in the lavage fluid readily and removal of these exfoliating cells directly from the mucosa is a matter of technique and perseverance.

Preparation of Smear:

Digestive juices and gastric acid cause deterioration of the cells; any delay in preparation deletes definite cellular characteristics.

The satisfactory lavage specimen will contain gross particles of tissue,

flecks of blood, or will be somewhat opaque. Direct smears may be made from such particles or from the flecks of blood to which malignant cells frequently seem attached. (When the lavage fluid appears merely opaque, direct smears have been of no value.)

There are two ways of handling the remaining lavage fluid.

1. It may be fixed immediately with ether-alcohol.
2. The fresh material may be immersed in an ice bath and processed within twenty minutes.

Use of one or both of these methods is determined by the cytotechnologist. In this laboratory immediate fixation has given excellent results. It is probably wise to use immediate fixation for specimens which are strongly acid in order to minimize cell deterioration. However, blood-tinged specimens are processed from the fresh material.

The fluid is centrifuged (2000 rpm for 10 min.) and the sediment smeared between two slides which are then placed in 95 percent alcohol or ether-alcohol fixative.

Staining:

Staining of slides is an art. A well-stained slide facilitates interpretation and helps standardize cell appearance and deviations. The cytotechnologist must understand the staining procedures thoroughly. Interest in the final result stimulates care and precise handling of the slide and smear.

Smears are stained according to the technique of Papanicolaou, detailed procedure for which may be found in any one of several histological or cytological texts. Adjustments in procedure may be made to meet certain deviations from the average (i.e. thickness of smear).

For gastric smears an initial dip for one minute in a mixture of 0.5 percent celloidin in ether-alcohol will tend to keep the thick gastric material adherent to the slide. Harris hematoxylin without acid is recommended for the nuclear stain. Because interpretation depends largely on intranuclear detail, the hematoxylin should be checked frequently for concentration so that the nuclei will not be overstained. (If intracellular detail can be seen in the polys, the stain should be satisfactory.) Two drops of ammonium hydroxide in 100 cc 70 percent alcohol has been used effectively instead of the lithium carbonate solution. While adequate washing after hematoxylin is one of the most important steps, use of running water is not advisable as this may prove too vigorous for some gastric smears. Using a series of water baths with careful transfer of slides will do the job well.

Alcohols and stains should be fresh, clean, and without residue. Daily filtering and changing is necessary. Contamination is a likely possibility in the case of heavy gastric smears. Detachment of cells or groups of cells from the slide will contaminate the solutions and may lead to false interpretation of a subsequent slide.

While still moist with xylol, the slide is mounted with neutral Canada Balsam using a 24 x 50 #1 cover slip and dried quickly on a electric hot plate. Excessive heating must be avoided. When the balsam bubbles, the slide should be removed, the excess balsam squeezed out, and the slide allowed to cool. This quick drying process allows the smear to be read and marked immediately.

Screening:

The entire smear must be screened and any questionable, suspicious, or characteristically malignant cells marked with India ink. This locates the area permanently and the cells may be easily found for reveiw.

THE CYTOLOGICAL APPEARANCE OF THE GASTRIC CELL

A satisfactory smear for cytological examination should

1. Contain well-stained gastric mucosal cells occurring singly, in sheets, layers, or palisades.
2. Contain well-preserved squamous cells.
3. Show color differentiation.
4. Show definite nuclear detail.
5. Be thin enough that details are not obliterated by depth or overlapping.

Normal gastric cells are columnar epithelial cells with adequate cytoplasm. In large sheets the cytoplasm often presents a honeycomb appearance. The nucleus is basal and consists of a network of chromatin, a small nucleolus, and a well defined nuclear membrane without chromatin condensation at the periphery. Even in the absence of cytoplasm, the isolated nuclei can usually be identified. Because gastric columnar cells are subjected to degeneration, cytologists must become familiar with the various stages of preservation of the normal exfoliating columnar cells.

The morphology of these cells changes markedly with disease and the malignant gastric cell presents a combination of characteristics. Groups of malignant cells show crowding, irregular arrangement, anisocytosis, poikilocytosis, uneven staining, and asymmetry. Malignant cells in such groups seem to elbow each other out of the way and can be readily identified. The single malignant cell is often more difficult to recognize. It may show an increased nuclear cytoplasmic ratio, an enlarged and hyperchromatic nucleus, and irregular, thickened nuclear membrane. The chromatin of malignant cells is increased and irregular, occurring in strands and in coarse uneven clumps. Mitotic figures are sometimes seen. Cellular borders may coalesce and the cells appear multinucleated. Malignant gastric cells may phagocytize other malignant cells,[9] leukocytes, debris, or erythrocytes.

In addition to gastric mucosal cells one may find:

1. *Squamous epithelial cells* which are probably washed down from the esophagus or mouth. Several variations in appearance of these cells have been described elsewhere.[11,12]
2. *Respiratory epithelial cells.* These are sometimes confusing but they are usually more slender than the columnar cells from the gastric mucosa. Occasionally the nucleus may appear a little more "active" than normal gastric cells and the nucleoli a little more prominent. Usually the cilia and the end plate can be seen.
3. *Erythrocytes.* These are readily distinguished by morphology and stain affinity. Our experience has not always confirmed Panico's statement[9] that, "The erythrocyte is rarely seen in a benign gastric smear. The presence of intact, crenated, or hemolyzed erythrocytes usually suggests a malignant stomach containing a friable vascular tumor."

4. *Leukocytes.* These cells may appear as (a) neutrophils, (b) lymphocytes, (c) plasma cells, (d) monocytes.
5. *Histiocytes.* These cells seem to cause difficulty in interpretation many times. There appear to be four types of histiocytes found in gastric smears. The normal or usual histiocyte has a small, round, oval, or bean-shaped vesicular nucleus which is eccentrically located in "Swiss cheesy" or "sudsy" appearing cytoplasm. A second type is larger with phagocytized dust or carbon particles. The third type of histiocyte presents a variety of odd shapes and nuclear activity. It is this third type which is sometimes difficult to differentiate from a malignant cell. The nucleus is hyperchromatic and presents a distorted appearance and often a vacuolated cytoplasm. A fourth type is represented by those large cells described by Graham and Rheault[5] and found in smears of untreated pernicious anemia patients. They are enlarged, atypical, and phagocytic.
6. *Goblet cells.* These are epithelial cells which exfoliate readily. The mucus which collects within the cytoplasm pushes the nucleus out of the way and gives a unipolar appearance to the cell. They are found in the stomach with atrophic gastritis.[15]
7. *Bacteria.* Because the malignant or pre-malignant stomach is often achlorhydric, bacteria are free to grow rapidly and profusely. Lacto-bacilli are frequently seen. Presence of tetrads has been considered significant because of their frequent appearance in the malignant smear. However, no definite correlation has yet been established.[13]
8. *Vegetable cells.* Remnants of food particles may be recovered. One case of Giardia lamblia was reported from the duodenal washings of a patient at this hospital.[17]
9. *Yeast cells.* If encountered, these should cause little difficulty in identification.

Interpretation:

Diagnostic impression is usually reported in a manner similar to that of vaginal smears: (1) no malignant cells identified, (2) atypicalities without recognizable evidence of malignant cells, (3) suspicious, doubtful or questionable cells seen; repeat examination advisable, (4) areas highly suspicious of but not conclusive of malignancy, (5) unequivocal tumor cells seen.

Some workers, however, prefer to use but three classifications for reporting gastric smears (1, 3, and 5).

THREE INTERESTING CYTOLOGICAL STUDIES

The Pernicious Anemia Patient

One of the promising facets in the study of gastric cytology at the Boston City Hospital is done in conjunction with the "PA" clinic where patients with this disorder have been closely followed for more than twenty-five years. The high rate of gastric cancer in patients with pernicious anemia (10%+)[14] warrants this periodic examination. Repeated cytological studies of these patients may reveal malignant cells at their earliest appearance. The lack of hydrochloric acid in the stomach of

pernicious anemia patients permits the recovery of well preserved gastric cells for study. This may be why the "PA" cells were one of the first variables of gastric cytology to have been recognized and classified.

"PA" cells sometimes have been incorrectly called tumor cells. However, the recent recognition of persistent typical changes brought about a revision of cytologic criteria for malignancy in pernicious anemia patients. The cytotechnologist must apply special criteria when studying gastric smears from these patients. Although the "PA" cells are usually intact and do not have the bizarre contour of some malignant cells, many of the nuclei demonstrate such activity that it is easy to see how these cells might have been confusing. Massey and Klayman stated, "Some 'active' cells of pernicious anemia can be considered as abnormal as columnar cells can appear and still remain non-malignant."[7]

It is this writer's impression that the epithelial cells from the pernicious anemia mucosa look as though they had been "working hard." Some are twice the normal size; the nuclear membrane is often folded; and the nucleoli are prominent. Others have a bland exhausted appearance. These are all in contrast to the uncluttered healthy appearance of normal gastric mucosal cells.

The Use of Cytometrics

Nuclear size is so often a feature in recognizing normal cells, regenerative cells, "PA" cells, and malignant cells that the use of cytometrics is indicated. Studying the actual size of the nuclei as suggested in the study conducted in 1956 at this laboratory offers potential usefulness as an adjunct to the diagnosis of gastric malignancy. "The nuclei of patients with pernicious anemia are larger and more variable than are those from normal gastric mucosa; the nuclei of the gastric cells from patients with proven cancer are still larger and even more variable."[4]

The Gastric Ulcer Problem

Repeated cytological examination of gastric ulcer patients may be used to substantiate clinical findings of benignancy or a growing clinical suspicion of malignancy. A study is being carried out in this laboratory to evaluate the use of cytology in gastric ulcer differential diagnosis.[16]

Summary

1. Exfoliative cytology is a challenging phase of clinical and investigative laboratory study and has as its aim the early detection of cancer.
2. Gastric cytology is a specialized study best carried out by trained cytologists working in the gastrointestinal unit of a hospital.
3. Methods for the collection of the gastric specimen, preparation of the smear; identification of the cells, and interpretation are outlined.
4. The pernicious anemia patient deserves special and vigilant attention because gastric carcinoma is an expected complication.
5. The value of gastric cytology as a pre-surgical diagnostic aid should be further evaluated.

Acknowledgment

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STUDIES TO FIND AN INHIBITOR-FREE MEDIA FOR SENSITIVITY ON THE SULFONAMIDES*

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With the increased number of antibacterial agents in use, singly and in combination, the need for a media on which one may accurately test the relative effect of different specific antibacterial agents on bacteria by the disc sensitivity method has become more apparent. A media of this type would be of great value to the clinical laboratory as it would enable the bacteriologist to perform disc sensitivity tests on the sulfonamides and the common antibiotics simultaneously.

Due to the antagonism to sulfonamide activity demonstrated by a variety of substances such as peptones and muscle extracts (Lockwood, 1938), a satisfactory media for performing sulfonamide sensitivities has not been made generally available for use in the clinical laboratory. This antagonism to the sulfonamides has been demonstrated to be paraminobenzoic acid and/or its analogues (Wood, 1940). It is obvious that the common bacteriological media which contain one or more of the antagonistic substances are unsatisfactory for testing the bacteriostatic action of sulfonamide drugs. Therefore, the author has endeavored to adapt an inhibitor-free media suitable for disc sensitivity tests on the sulfonamides and other common antibiotics.

Since the discovery of sulfanilamide (Trefouel et. al., 1935), many experiments have been carried out in an attempt to find an inhibitor-free media. Pour plates of glycerine-agar, free of meat infusion, with 5% horse blood added was used by Lockwood in his studies of the mechanism of action of sulfanilamide (Lockwood, 1938). This is a very simple and inexpensive medium to prepare, but horse blood contains chemically undefined constituents which present a problem of procuring standardized blood.

MacLeod demonstrated the inhibition of bacteriostatic drugs by a number of substances of animal and bacterial origin (MacLeod, 1940) by using a standard synthetic media previously described (Sahyun et. al., 1936). For growth of certain pathogenic bacteria, the addition of fresh beef liver infusion was necessary. This was obtained from a freshly slaughtered calf and processed immediately; consequently, this inconvenience would seem to make this medium impractical for routine use. However, this appears to be a satisfactory media since it supports the growth of common pathogenic bacteria and is considered free of antagonists to sulfonamides.

The addition of 5%, or less, of lysed horse blood was shown by Harper and Cawston (1945) to neutralize the antagonizers present in nutrient broth and different types of agar. The procedure of adding lysed horse blood to eliminate this problem is very desirable, since many different types of media may be used. However, procuring standardized horse blood may offset the simplicity of this technique.

Adams and Roe (1945) used the semi-synthetic medium of Bernheimer et. al. (1942) for the cultivation of pneumococci. They substi-

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tuted an acid hydrolysate of Harris' vitamin-free casein as a base and added choline and asparagine instead of the acid digest of technical casein used for streptococci by Bernheimer. This medium was found to be inhibitor-free and supported the growth of all the common pneumococci. Adams and Roe showed that with the addition of 2.5% rabbit serum (found to be free of inhibitors by MacLeod in 1940) this media supported the growth of all the group A streptococci. It is the author's belief that this medium is the most promising as it can be prepared free of inhibitors to sulfa drugs and probably will support the growth of all common pathogenic organisms.

MATERIALS AND METHODS

Of the previously described medium, the semi-synthetic medium of Adams and Roe was selected for the test. This medium is composed of and made up as follows:

BASAL MEDIUM—FOR ONE LITER OF MEDIUM

Acid Hydrolysate of casein	200 ml. of 10% solution
1-Cystine	150 mg.
1-Tryptophane	20 mg.
KCl	3 gm.
$Na_2HPO_412H_2O$	7.5 gm.
$MgSo_47H_2O$	0.5 gm.
Distilled water to make	900 ml.

Adjust pH to 7.5, heat to boiling, filter, and tube in 9 ml. amounts or appropriate multiple. Autoclave at 15 lb. pressure and 121° C Temperature for 15 minutes.

SOLUTION I—VITAMIN MIXTURE FOR 12.5 LITERS

Biotin	0.015 mg.
Nicotinic acid	15.0 mg.
Pyridixine	15.0 mg.
Calcium pantothenate	60.0 mg.
Thiamine	15.0 mg.
Riboflavin	7.0 mg.
Adenine sulfate	150.0 mg.
Uracil	150.0 mg.

Dissolve in 100 ml. of distilled water and sterilize by filtration. Store in refrigerator.

SOLUTION II—SALT MIXTURE FOR 50 LITERS

$FeSO_4 \cdot 7H_2O$	50 mg.
$CuSO_4 \cdot 5H_2O$	50 mg.
$ZnSO_4 \cdot 7H_2O$	50 mg.
$MnCL_2 \cdot 4H_2O$	20 mg.
HCL Concentrated	1 ml.

Dissolve in 100 ml. of distilled water and sterilize by boiling 10 minutes.

ADDITION MIXTURE PER LITER OF MEDIUM

Vitamin mixture (Solution I)	8.0 ml.
Salt mixture (Solution II)	2.0 ml.
Glucose (20% Solution)	10.0 ml.
Glutamine	200.0 mg.
Asparagine	100.0 mg.
Choline	10.0 mg.
$CaCl_2 \cdot 2H_2O$	10.0 mg.
Distilled water to make	50.0 ml.

Sterilize by filtration and store in refrigerator. Add 0.5 ml. to each 9 ml. of basal medium. This addition mixture should not be kept longer than a few weeks as the glutamine is unstable. Solutions I and II appear to keep indefinitely.

BICARBONATE—THIOGLYCOLLATE MIXTURE

Thioglycollic acid 10%

Add 1 ml. of thioglycollic acid to 9 ml. of sterile distilled water, mix well, and heat in boiling water bath for ten minutes.

Bicarbonate. Weight 200 mg. samples of sodium bicarbonate into a test tube and autoclave. Add 10 ml. of sterile distilled water to a test tube containing bicarbonate and dissolve the latter. Then add 0.2 ml. 10% thioglycollic acid and mix well immediately. Add 0.5 ml. of the mixture to each 9.5 ml. of medium. This bicarbonate-thioglycollate mixture is unstable and must be made up and added to medium just prior to inoculation.

The control or reference medium chosen was an inhibitor-free medium used as a basic medium for the study of inhibitors by MacLeod (1940). This medium is chemically defined.

Brain-Heart Infusion (Difco) without the addition of para-aminobenzoic acid was used as a reference medium containing inhibitors to demonstrate the difference in inhibition of antibacterial action in the presence of inhibitors as compared to that action in the absence of inhibitors.

Bacto-Mueller Hinton Medium (Difco) is the medium of choice for performing sulfa sensitivity tests in many laboratories at the present time; therefore, it has been incorporated into this study for a comparison with the test medium. According to Difco Laboratories, this medium contains a minimum amount of antagonists to sulfa drugs.

Since the sulfonamides are all derivatives of sulfanilamide and were shown to react similarly toward inhibitors in various medium (White et. al., 1941), the results obtained with Gantrisin should be representative of this group of chemotherapeutic agents. It also has a similar degree of effectiveness in vitro against micro-organisms as compared with other sulfonamides on various medium, Gantrisin, (3, 4 dimethyl-5-sulfanilamide-isoxazole) because of its availability in impregnated paper discs of varying concentrations was, therefore, used as the test sulfonamide.

The test organism used in this study was a clinical strain of Escherichia coli isolated from a genito-urinary infection.* This organism was found suitable since it was susceptible to Gantrisin and gave luxuriant growth on the chemically defined medium of MacLeod which was used as a reference medium.

PROCEDURE

Liquid Phase

For uniformity of results, the following precautions were taken in the liquid phase.

1. Sufficient medium for performing the full course of tests was prepared originally.
2. The final pH of each medium was checked with a Beckman pH meter.
3. One standard loop of the test organism was transferred from MacConkey's agar to 5 ml. of MacLeod's liquid medium and incubated at 37° C. for 15 hours; then one standard loop of this growth was transferred to 5 ml. of MacLeod's liquid medium and incubated for the next day. This eliminated the possibility of carrying inhibitors over into the test with the test organisms.
4. All dilutions of Gantrisin in each separate medium were prepared from the same original stock solution of Gantrisin.
5. Exactly 5 ml. of each concentration of the three respective media was pipetted into the same size test tube.
6. One standard loop of test organisms was inoculated into each tube.
7. MacLeod's inhibitor-free medium was included in each test as a control.
8. All glassware to be used was rinsed with distilled water and sterilized in the autoclave.

In this phase of the problem, the relative amount of inhibitor present in Brain-Heart infusion and the test medium as compared with the amount present in MacLeod's medium was determined by the test tube technique. This technique was performed as follows: Using sterile equipment, 500 ml. quantities of MacLeod's, Brain-Heart Infusion, and the test media were prepared in the liquid state as previously described. Stock solutions of Gantrisin were then prepared in each medium by diluting 1 ml. of the original stock solution of Gantrisin as diethylamine salt to 100 ml. with each medium giving stock solutions containing 400 mg. % Gantrisin. From these stock solutions in each medium, the following concentrations of Gantrisin were prepared by dilution with more medium: 10,000 mcg., 5,000 mcg., 1,000 mcg., 250 mcg., 10 mcg., 3 mcg. For the test medium it was necessary to prepare this stock solution and dilutions in the basal medium of this medium since the addition and bicarbonate-thiglycollate mixtures are not added until just prior to inoculation. Sufficient Gantrisin was added to each 9 ml. of basal medium to give the desired concentration after 0.5 ml. of addition mixture and 0.5 ml. of bicarbonate-thioglycollate mixture were added.

Exactly 5 ml. of all concentrations of the three media was pipetted with sterile pipettes into sterile 12 x 100 mm. test tubes and labelled

* This strain of Escherichia coli was isolated from a patient with a genito-urinary infection at Firmin Desloge Hospital, 1954.

with wax pencil. One standard loop of a 15 hour growth of the test organism, roughly estimated by pour plate colony count at 80,000 to 120,000 organisms, was added to each tube. An inoculum control and medium control was also set up on each medium by pipetting 5 ml. of plain medium into two separate tubes. One standard loop of test organism was added to each inoculum control. All tubes were then incubated at 37° C. for 15 hours after which the degree of inhibition of growth of the test organism was read macroscopically and reported according to the degree of turbidity present. MacConkey's agar plates were then streaked with one standard loop of medium from all tubes showing inhibition of the test organism for an estimate of the viable organisms present. This exact procedure was repeated four consecutive days. Results are recorded in Table I.

Table I

RESULTS OF LIQUID MEDIA FINDINGS

MEDIUM	Concentration of Gantrisin	Days			
		1st	2nd	3rd	4th
MacLeod's	10,000 mcg.	4+	4+	4+	4+
	5,000 mcg.	4+	4+	4+	4+
	1,000 mcg.*	4+	4+	4+	4+
	250 mcg.*	3+	3+	4+	4+
	50 mcg.	0	0	0	0
	10 mcg.	0	0	0	0
	3 mcg.	0	0	0	0
Test organism control	0 mcg.	Heavy growth			
Medium control	0 mcg.	No growth			
Test medium	10,000 mcg.	4+	4+	4+	4+
	5,000 mcg.	4+	4+	4+	4+
	1,000 mcg.*	3+	3+	4+	4+
	250 mcg.*	2+	3+	3+	3+
	50 mcg.	0	0	0	0
	10 mcg.	0	0	0	0
	3 mcg.	0	0	0	0
Test organism control	0 mcg.	Heavy growth			
Medium Control	0 mcg.	No growth			
Brain-Heart Infusion	10,000 mcg.	0	0	0	0
	5,000 mcg.	0	0	0	0
	1,000 mcg.	0	0	0	0
	250 mcg.	0	0	0	0
	50 mcg.	0	0	0	0
	10 mcg.	0	0	0	0
	3 mcg.	0	0	0	0
Test organism control	0 mcg.	Heavy growth			
Medium control	0 mcg.	No growth			

4+—Complete inhibition of visible growth.
3+—¾ inhibition of visible growth as compared to test organism control.
2+—½ inhibition of visible growth as compared to test organism control.
0—Visible growth equal to the test organism control tube.

*Gross observation showed the test medium to be more turbid in these concentrations than MacLeod's, but by streaking on MacConkey's agar, it was shown that the test medium contained the same number or less viable organisms than MacLeod's, thereby indicating that this turbidity may have been due to the bacteriacidal action of Gantrisin after rapid growth had taken place.

Solid Phase

The same strain of Escherichia coli used in the liquid phase was used in the solid phase for uniformity of results and comparison. One standard loop of this organism was transferred daily to 5 ml. of MacLeod's liquid media to prevent the transfer of inhibitors with the test organism. For inoculation of agar plates, a 1-100 dilution was made of this 15 hour growth of organisms in physiological saline, then 0.5 ml. of this dilution

was pipetted onto the surface of each plate with a sterile pipette and spread evenly over the surface.

Gantrisin sensitivity discs (Difco) of 250 mcg., 50 mcg., and 10 mcg. were used in this test. Gantrisin sensitivity disc (Desi disc) of 1000 mcg. was also included. Only the above mentioned concentrations of Gantrisin impregnated paper discs were tested since other concentrations were not commercially available at the time of this work.

Bacto-Mueller Hinton medium was included with MacLeod's, Brain-Heart Infusion and the test medium for comparison as it is the media of choice at the present time in most clinical laboratories for performing sulfa sensitivity tests on solid agar plates.

MacLeod's, Bacto-Mueller Hinton, and Brain-Heart Infusion media were made up in 250 ml. quantities and 1.5% agar was added to each; then placed in a boiling water bath to dissolve the agar, stoppered with cotton and autoclaved at previously specified pressure and time. In the preparation of the test medium, 3.75 gm. of agar was added to 225 ml. of basal medium, the desired concentration of 1.5% being obtained after autoclaving, when the bicarbonate-thioglycollate acid mixture and the addition mixture were added. Due to the instability of the bicarbonate-thioglycollic acid mixture and the fragility of the addition mixture, these two solutions cannot be added until just prior to pouring the plates. After all flasks had been removed from the autoclave, they were placed in hot water, 20 ml. aliquots of each media was pipetted with a sterile pipette into 20 x 150 mm. test tubes and stored in the refrigerator. Only 18 ml. quantities of the basal-agar medium were transferred to tubes as the bicarbonate-thioglycollate mixture and addition mixture must still be added to each.

For preparation of plates, one tube of each medium was removed from the refrigerator and heated in a boiling water bath to dissolve; they were then placed in a 60° C. water bath to keep them from solidifying. These tubes were removed from the water bath and poured directly into 15 x 95 mm. sterile petri dishes, placed on a flat surface, and allowed to solidify.

Before pouring, the basal-agar medium was allowed to cool approximately 50° C., 1 ml. addition mixture and 1 ml. of bicarbonate-thioglycollate mixture were added; it was then rotated to mix the solutions and poured into sterile 15 x 95 mm. petri dishes.

Exactly 0.5 ml. of a 1-100 dilution in physiological saline of a 15 hour growth of the test organism was pipetted with a sterile pipette onto each plate and spread evenly over the surface. One disc of each of the four concentrations of Gantrisin used was then spaced evenly on the surface of each respective plate, using sterile tweezers which were flamed between the transfer of each disc. All plates were then incubated at 37° C. for 15 hours. After removal from the incubator, the zone of inhibition was measured with a mm. ruler by measuring from the edge of the sensitivity disc to the outer limits of the zone of inhibition of all discs demonstrating this characteristic. This procedure was repeated three consecutive times, all techniques being kept as nearly uniform as possible; that is to say, conformity in the size of the petri plates, amounts of agar used.

time of incubation, approximate number of organisms used, as well as conformity in all other methods and materials used. Results are recorded in Table II according to the size of the zones of inhibition observed.

Table II
RESULTS OF SOLID MEDIA STUDY

MEDIUM	Concentration of Gantrisin	ZONE OF INHIBITION IN MMS		
		1st Test	2nd Test	3rd Test
MacLeod's	10 mcg.	6 mm.	8 mm.	10 mm.
	50 mcg.	14 mm.	14 mm.	19 mm.
	250 mcg.	20 mm.	20 mm.	21 mm.
	1000 mcg.	20 mm.	22 mm.	25 mm.
Test Medium	10 mcg.	1 mm.	0 mm.	0 mm.
	50 mcg.	10 mm.	8 mm.	7 mm.
	250 mcg.	16 mm.	16 mm.	17 mm.
	1000 mcg.	17 mm.	20 mm.	19 mm.
Bacto-Mueller Hinton	10 mcg.	0 mm.	0 mm.	0 mm.
	50 mcg.	2 mm.	2 mm.	4 mm.
	250 mcg.	8 mm.	8 mm.	10 mm.
	1000 mcg.	9 mm.	9 mm.	10 mm.
Brain-Heart Infusion	10 mcg.	0 mm.	0 mm.	0 mm.
	50 mcg.	0 mm.	0 mm.	0 mm.
	250 mcg.	0 mm.	0 mm.	0 mm.
	1000 mcg.	0 mm.	0 mm.	0 mm.

1st test—500,000—600,000/0.5 ml. inoculum (pour plate estimate).
2nd test—500,000—600,000/0.5 ml. inoculum (pour plate estimate).
3rd test—400,000—500,000/0.5 ml. inoculum (pour plate estimate).

DISCUSSION

As reported in Table I, Brain-Heart Infusion broth did not show inhibition of growth in any of the four tests performed using seven concentrations of Gantrisin ranging from 3 mcg. to 10,000 mcg. %. The test organism control with this media gave a heavy growth and the media control showed no growth.

The test media failed to give inhibition of growth in tubes containing less than 250 mcg. % of Gantrisin. Only partial inhibition of growth was observed at 250 mcg. % and partial to complete inhibition of growth in concentrations above 250 mcg. %. The test organism control in this medium also showed heavy growth while the medium control gave no growth.

Heavy growth of the test organism control was demonstrated on MacLeod's liquid medium. The medium control showed no growth. The test organism was not inhibited in this medium with a concentration of Gantrisin below 250 mcg. %. Partial to complete inhibition was noted at 250 mcg. % and partial to complete inhibition was observed in concentrations above 250 mcg. %. These results were substantially duplicated on each of four consecutive days.

The test organism was not inhibited on Brain-Heart Infusion agar by any of the four concentrations of Gantrisin discs used. On MacLeod's media a large zone of inhibition was observed around all four concentrations of Gantrisin discs ranging from a 6 mm. zone around the 10 mcg. disc to a 25 mm. zone around the 1,000 mcg. disc. Zones of inhibition on the test media ranged from no zone around the 10 mcg. disc to a 20 mm. zone around the 1,000 mcg. disc. Zones of inhibition ranging from no zone around the 10 mcg. disc to 10 mm. around the

1,000 mcg. disc were obtained with Bacto-Mueller Hinton medium. These results are illustrated in Table II.

As evidenced by the results tabulated in Tables I and II, MacLeod's media has shown a more marked inhibition than the test media. This may be explained by the fact that with MacLeod's, being a more simple media, the lag phase of the test organism was longer allowing Gantrisin to diffuse into the surrounding media.

CONCLUSION

Inhibition of growth was not demonstrated in either the liquid or solid media using Brain-Heart Infusion although concentrations of Gantrisin ranging from 3 mcg. to 10,000 mcg. were used. This agrees with work performed by previous investigators which showed that substances contained in this media inhibit the action of the sulfonamides.

A comparison of the test media with MacLeod's inhibitor-free media in the test tube technique gave essentially the same results. This indicates that this media is free of inhibitors as stated by other investigators.

Bacto-Mueller Hinton media has been shown in these studies to be superior to the routine laboratory media such as Brain-Heart Infusion for studying sulfa drug sensitivities, but two other media have given more remarkable results. Both MacLeod's and the test media are simply prepared and both have been demonstrated to be superior to Bacto-Mueller Hinton. While MacLeod's has given ideal results in this study using *Escherichia coli* as the test organism, it would not support the growth of more fastidious organism. With these considerations, the author feels that the test media, the Pneumococcus Media of Adams and Roe, should represent the media of choice.

Summary

The object of this study was to find an inhibitor-free medium suitable for performing sensitivity tests on the sulfonamides. A liquid medium has been described, adapted for use as a solid medium, and shown to give comparable results with the inhibitor-free medium of MacLeod by the test tube and paper disc techniques. While the author believes that suitable media were included for controls, further tests including a wide variety of sulfonamides and test organisms should be made to verify the indications here demonstrated.

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A MICROBIOLOGICAL STUDY OF PEDIATRIC VAGINITIS

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A study group of patients of age 3 months to 12 years with a median age of 7.3 years and a control group of age 6 months to 14 years, with a median age of 6 years were examined. Efforts were made to determine the etiology of vaginitis and the changes in the prevalence of the organisms noted when an extrogenic substance was used.

The children were from families of low economic status. Therefore the findings cannot be applicable to a total population. Instructions for application of the cream containing estrogen were not followed consistently. The conclusions must be understood with that in mind.

21% of the patients gave a positive culture of Neisseria gonorrheae which was supported by the chi-square test as the agent responsible for the vaginitis. Those who had topically applied the estrogenic cream to the vulvar region did not continue to show the gonococcus. These patients developed and adult-type of vaginal epithelium along with the Döderlein's bacilli and a pH of 4 which reverted to the normal premenarchal condition and pH of 7 after treatment. 5.2% showed Trichomonas vaginalis—the controls did not. The estrogenic cream had no effect on this organism. The study group and controls showed the similar presence of coagulase-positive strains of M. pyogenes var. aureus which could not be correlated with the observed vaginitis. Enteropathogenic strains of E. coli and H. vaginalis were not recovered from any of the premenarchal patients. "Nonspecific" vaginitis patients and normal controls equally showed a large number of various bacteria. The "nonspecific" type of vaginitis may be due to some viral agent e.g., the echo, adenovirus, Coxsackie or an unknown virus.

Primary microbiological methods used: scotch tape perianal for possible Enterobius vermicularis; saline suspensions for microscopical Trichomonas vaginalis and for inoculation on Sabouraud's glucose agar; primary Gram stained smears; direct inoculation of cotton swab obtained specimens of Difco GC basic medium with hemoglobin and supplement A with subsequent incubation at 37° C in a candle jar containing about 10% CO_2; another cotton swab obtained specimen immersed in tube with about 1 ml. of proteose peptone No. 3 broth for about an hour before subculturing to: blood agar plates; Haemophilus vaginalis medium and Casman's medium; GC plate followed by oxidase reaction of suspicious colonies and subsequent confirmation by sugar reactions; tomato agar at pH 5 and Rogosa's medium to demonstrate Döderlein bacillus and yeasts; plain nutrient agar plates; Difco PPLO agar with 15% horse serum with first cases; routine enteric media and Escherichia coli isolates tested for agglutination with enteropathogenic E. coli antisera (Lederle); Thioglycollate broth and NIH thioglycollate broth for possible anaerobes; Douglas broth; coagulase broth. Washings with Hank's balanced salt solution were frozen for subsequent viral investigation. Exfoliated cells identified and estrogenic effects determined. D'Antoni's solution used to identify glycogen. pH determined with nitrazine and hydrion papers (range 4-9).

MBC

MECHANISM OF HEMATOPOIESES—HEMATOPOIETIC EFFECTS OF WHOLE HUMAN AND BOVINE SERUM

Bernard Steinberg, et al.
(Toledo Hosp. Inst. of Med. Research, Toledo, Ohio)
Lab. Invest. 7, 458-67 (1958)

Observations made of hematologic effect of multiple injections, detoxification of serum injections and timing of injection intravenously of human and bovine serum into 95 rabbits. After multiple intravenous injections the rabbit bone marrow showed inhibition of maturation and occasionally suppression of hematopoiesis. The antigen-antibody reaction in which the marrow is the shock organ and the "toxic effect" of foreign proteins on the marrow were suggested as explanations for the action of sera on the marrow. Third explanation favored by authors was that specific factors concerned with the regulation mechanism of hematopoiesis exists in the serum.

A NEW ANTIBIOTIC ASSAY MEDIUM FOR *ENTEROBACTERIACEAE* AND RELATED GRAM-NEGATIVE RODS†

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The commonly used culture medium in antibiotic sensitivity testing by the agar diffusion technique is blood agar (BA). A disadvantage of this medium lies in the tendency of many bacterial strains among *Enterobacteriaceae* and related Gram-negative rods to overgrow rapidly initial inhibition zones around any of the discs employed, making the interpretation of the results of antibiotic assays difficult and, upon prolonged incubation, often impossible. In the case of sulfa compounds, this phenomenon was ascribed to the competitive inhibition of *p*-aminobenzoic acid (PABA).[5] Muller-Hinton agar (MH), a medium free of PABA, was recommended for determining bacterial susceptibility to sulfa drugs.[1] In our hands, this medium proved unsatisfactory.

The studies of several investigators[3,4] demonstrating inhibition of sulfa drug bacteriostasis by peptones, amino acids, and various other organic nitrogenous compounds led us to explore the possibility that by reducing the amount of such substances in the culture medium clear inhibition zones free from overgrowth might be obtained. A synthetic base blood agar (SBBA) was prepared. The following ingredients were dissolved in 1 L. of distilled water.

KH_2PO_4	3.53 gm.*	$MgSO_4$	0.10 gm.
$NaNH_4HPO_4 \cdot 4H_2O$	5.58 gm.*	Glucose	1.00 gm.
NaCl	5.00 gm.	Agar	15.00 gm.

* This salt mixture yields a M/15 phosphate buffer of pH 7.

To this basal mixture, autoclaved and cooled to 45° C., 10 percent sheep's blood was added.

The complete medium supported well the growth of *Enterobacteriaceae* and related Gram-negative rods, but only poorly, if at all, that of Gram-positive cocci. The data presented in Table 1, illustrative of many others

TABLE 1

Effect of Time of Incubation on Zones of Growth Inhibition of a Strain of *Aerobacter aerogenes*, Using Blood Agar (BA) and Synthetic Base Blood Agar (SBBA)

Antibiotic	Time of Incubation (hrs.)	Inhibition Zone* (mm)	
		BA	SBBA
Neomycin	4	4-clear	slight growth
	6–24	overgrown**	7-clear
Polymixin B	4	3-clear	slight growth
	6–24	overgrown**	6-clear
Kantrex	4	5-clear	slight growth
	6–24	overgrown**	8-clear

* Distance from edge of antibiotic disc to boundary of bacterial growth.
** The recognizable zone of initial inhibition decreased in size upon prolonged incubation.

† Received for publication September, 1958.

obtained, offer a comparison of blood agar (BA) prepared with blood agar base (Difco) and SBBA in antibiotic assays. For inocula, 1:10 and 1:100 dilutions in distilled water of a 24 hour brain heart infusion broth culture of *Aerobacter aerogenes* were used. The plates were seeded by swabbing them with the bacterial suspensions. Antibiotic discs (BBL) of low concentration only were placed on the seeded agar surfaces. Zones on SBBA remained sharply defined and unchanged in size even after 48 hours of incubation. While tenfold dilution had a negligible effect on the lag period of growth and the size of inhibition zones, 1:100 broth culture dilutions were employed in routine testing.

In Table 2, Muller-Hinton agar (MH) and SBBA are compared in testing the sensitivity of a strain of *Proteus mirabilis* to two sulfa compounds.

TABLE 2

Effect of Time of Incubation on Zones of Growth Inhibition of a Strain of *Proteus mirabilis* around Sulfa-Impregnated Discs, Using Muller-Hinton Agar (MH) and Synthetic Base Blood Agar (SBBA)

Sulfa Compound	Time of Incubation (hrs.)	Inhibition Zone (mm)	
		MH	SBBA
Gantrisin	4	0-uniform growth	slight growth
	6-24	7-overgrown	13-clear
Triple Sulfa	4	0-uniform growth	slight growth
	6-24	6-overgrown	11-clear

It is of interest that overgrowth of inhibition zones on MH and BA arises in different ways. On MH, growth is initially uninhibited and appears to become slower or arrested around the discs only after about 6 hours of incubation. A possible explanation might be sought in a recent study[2] demonstrating the counteracting effects of growth rates and diffusion rates on antibiotic assays by the agar diffusion technique.

Our experience for several months with SBBA as a medium for sensitivity testing has been favorable. We have found it particularly useful in antibiotic assays with strains of *Escherichia, Aerobacter, Proteus,* and *Pseudomonas.*

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THE ESTABLISHMENT OF A VIRUS DIAGNOSTIC LABORATORY IN A MEDIUM SIZED HOSPITAL*

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Introduction

In recent years practical methods in the study of viruses and their relation to human disease have permitted the emergence of virology from research laboratories into diagnostic laboratories There have been many reports on the serological approach to diagnostic virology,[1,2,3] the isolation of viruses on a clinical level,[2,4] and there has been an excellent review of the entire subject of virological technics.[5,6,7] These papers have originated in national or state laboratories, university medical centers or large research hospitals.

This report is of the establishment of a diagnostic virology laboratory in a medium sized hospital away from direct contact with a university or a medical research center and not supported by any grants or subsidies. The work performed has been purely routine in character with no purpose other than to satisfy the needs of our own medical staff and their patients. Initially only serological tests were offered, although appropriate isolation specimens were sometimes collected and preserved in the deepfreeze for use in familiarizing ourselves with viral isolation technics. As the confidence of the clinicians was gained and their interest awakened, tissue culture isolations were introduced, and finally, when the Asian influenza epidemic reached our area, embryonated egg technics were established.

Personnel

Although the work load in the virology department has now progressed to the point where the services of a full-time technologist are necessary, in the beginning the serology division was able to take care of the serological procedures, and the microbiologists took care of the tissue cultures and the isolations. Their success in adapting themselves to virological procedures has convinced us that anyone with previous laboratory training in microbiological and serological technics can, with a minimum amount of instruction, be able to carry out the technics required in diagnostic virology.

We were very fortunate in obtaining the services of a local virologist as consultant. The guidance of the virologist in the set-up of the laboratory, the choice of equipment, and in the teaching and demonstration of technics was invaluable, although his ultimate function, of course, was the interpretation of results, the choice of procedures to be introduced, and consultation with the staff doctors on problems in virological diseases.

Equipment of the Laboratory

The objective in choosing equipment was to obtain an adequately functioning laboratory without exceeding the thousand-dollar budget

* Read before the 26th Annual Convention of ASMT, Milwaukee, Wisconsin, June, 1958.

allotted to the inauguration of the program. A room within the laboratory suite was chosen. It is not a large room—twelve by fifteen feet—but it has suitable lighting, ventilation, gas and electrical outlets, and was close enough to the biochemistry department to share their centrifuge and icebox.

One counter type table with cupboard space and two desk type laboratory units were built by the hospital's maintenance department. All three were fitted with white glass tops which are both inexpensive and easily disinfected.

A sterile hood is an absolute necessity for the protection of the technologist from viruses, and the tissue cultures from bacteria. Commercially available isolation units were prohibitably expensive. After consultation with the virologist our engineer built one of stainless steel, pyrex glass, and an ultraviolet lamp according to the diagram prepared at their conference (Fig. 1). The cost of this unit was about a hundred dollars.

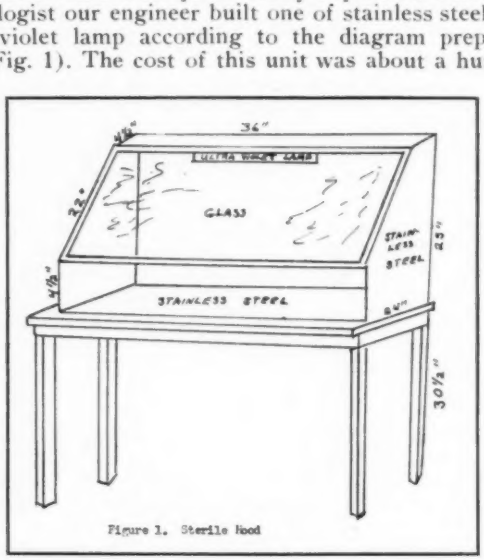

Figure 1. Sterile Hood

A deepfreeze unit was one of the major expenses. An upright Coldspot appliance of 18 cu. ft. capacity and capable of maintaining a temperature of —30° C was selected, although other makes would also be satisfactory. This type of appliance takes up relatively little space; the shelves and door compartments provide ready availability of stored materials; and local repairmen can service the unit if necessary.

A waterbath large enough to hold six to eight Wasserman type racks was procured from one of the supply houses. It has a cover and a thermostat operating at 37° C and 56° C.

The bacteriological incubator has a capacity of 6 cu. ft. and is well insulated. By the simple expedient of keeping a large pan of water in the incubator preset at 37.5° C, temperatures of 35° C to 36° C can be obtained for tissue cultures; while a small pan of water permits temperatures of 37° C for the incubation of embryonated eggs.

Several types of tissue culture racks are commercially available. The stainless steel racks provided with a spring to hold tubes in a stationary position* are probably the most practical although their initial expense is somewhat greater than that of other types. Three or four of the size accommodating 16 x 125 mm. screwtop pyrex culture tubes are desirable.

Selas bacteriological filters of porosity 0.02 were purchased. Probably other varieties of bacteriological filters are satisfactory, although the asbestos mat type tends to retain some virus by absorption. Six of these units were sufficient.

Cornwall pipets are not absolutely necessary, but the two ml. size has been of inestimable value in expediting the pipetting of reagents in the performance of complement fixation tests.

Two or three propipettes obtainable from most laboratory supply houses have been very useful in transferring infectious materials safely, a swell as for the addition of sterile solutions to media, cultures, and vials.

The only other equipment necessary was the usual allotment of rubber-lined screw top 16 x 125 ml. pyrex culture tubes, a few ordinary prescription bottles with rubber lined plastic screw tops; Wasserman and Kahn tubes and racks, pipettes, filter flasks, and screw-top vials for the preservation of isolation specimens in the deepfreeze.

Scope of Work Offered

1. Serological

One of the momentous decisions in the establishment of the virology department was just which tests should be offered the medical staff. Ultimately we concluded that the following listing should prove adequate for the majority of cases occurring in the locality and would serve as a beginning:

Complement fixation tests:
- Poliomyelitis, Types I, II, and III
- Lymphocytic choriomeningitis
- St. Louis encephalitis
- Eastern equine encephalitis
- Western equine encephalitis
- Lymphogranuloma venerum
- Mumps
- Psittacosis
- Adenovirus
- Influenza virus, A, B, and C

Neutralization tests:
- Poliomyelitis, I, II, and III
- Coxsackie Group B and A-9

Hemagglutination-inhibition tests
- Influenza strains, A, B, C, and Asian

Cold Agglutination
- (Agent of viral pneumonia)

* Obtainable from Drummond Company, 6022 Media Street, Philadelphia, Penn.

Antigens and antisera were obtained commercially* for the above series of tests. Lately we have been preparing our own antigens for the influenza, Coxsackie, adenovirus, and poliomyelitis tests as well as antisera for the latter.

The complement-fixation test has been of the greatest use because it has a wider application than the other systems. Probably any of the established sheep cell—rabbit amboceptor—guinea pig complement systems can be satisfactorily used as long as the laboratory standardizes it for viral and rickettsial antigens and antisera. We chose the modified Kolmer technic because it provides a happy balance between sensitivity and specificity, is easily adapted to viral diseases, and is familiar to most workers in the serological field.

The actual mechanics of the hemagglutination-inhibition test are so simple that it seems it should supersede the more tedious complement fixation technic. Perhaps it will do so, when more methods of removing naturally occurring inhibitors in human serum are discovered. We use the hemagglutination-inhibition test to detect strain specificity in the influenza virus infections; periodate treatment of the serum is instituted and then the serial dilutions of the serum are added to standardized doses of virus antigen, incubated, and subsequently sheep cells or human Group O erythrocytes are added.

The neutralization test employed in our laboratories is the usual one wherein the serum-dilutions are added to the medium of tissue culture cells, and the Coxsackie (or other) virus strains are innoculated.

In all the tests, except the cold agglutination, a serum sample was collected during the acute phase of the disease and frozen until the second (convalescent) serum was obtained two or three weeks later. Both serums were tested simultaneously, the acute serum serving as a base-line anti-body titer for comparison with the convalescent serum. In most instances a two-tube rise in titer was considered of diagnostic import.

In order to assure the collection of the convalescent serum we found it helpful to give the patient a little card explaining that the second sample was necessary to complete the tests, and that there would be no charge made for the "second test." Approximately two weeks later we mailed a form letter to those patients who had returned home as a reminder. Very few have failed to comply with the request.

2. Tissue Culture Isolations

From our experience with the serological tests we realized that the viruses we would most likely encounter in the locality were poliomyelitis, influenza, adenovirus, Coxsackie B group and A-9, and possibly the Echo group. The influenza virus would necessarily be isolated in embryonated eggs. As host cells for the other viruses we selected HeLa cells although we also experimented for some months with Hep-prostatic cancer cells, and a culture of normal heart cells. Any of the cell lines proved adequate and can be obtained from commercial sources** or from a neighboring virological or tissue culture laboratory.

* Lederle Laboratories Division, American Cyanamid Company, New York 20, N. Y. Markham Laboratories, Chicago 20, Illinois; and Microbiological Associates, Inc., Washington, D. C.

**Microbiological Associates, Inc., Washington, D. C.

Protocols for the maintenance of these cell lines in culture are available in a number of texts (9, 10) and in the literature. The technics are easily learned by a technologist who has had experience in bacteriological procedures, but it is better to at least see a demonstration than to try to "pick them up" by oneself.

Of the several media and balanced salt solutions described for the maintenance of cell lines, we chose Hank's balanced salt solution, Eagle's maintenance medium, Eagle's Nutrient Medium, and Lactalbumin hydrolysate medium (9) because they were more universal in their application to various cell lines and viruses. They can be purchased commercially* in concentrated form, or can be prepared in the laboratory. It is, of course, much less expensive to make one's own. The three media consist of Hank's balanced salt solution (9) easily prepared in the laboratory, to which is added amino acids*, 1-glutamic acid*, vitamins*, and either horse serum* or human serum which has been salvaged from the routine serology tests and sterilized by passage through a bacteriological filter. Penicillin, dihydrostreptomycin, neomycin, and mycostatin were added to all media.

Isolation specimens consisted of throat swabbings or washings in respiratory diseases and rectal swabbings or stool specimens in the encephalitis diseases; intestinal mucosa, lung and brain tissues have been submitted from the autopsy room. All specimens were immediately placed in three milliliters of lactalbumin hydrolysate or Eagle's Maintenance Medium, to which antibiotics had been added, and placed in the deepfreeze until processed. These were subsequently innoculated into HeLa cell cultures bathed in Eagle's Maintenance Medium; passed through three or four cell cultures to eliminate cytopathogenic effects from the toxicity of the inoculum; those displaying evidence of cytopathogenicity of viral origin were subjected to neutralization by appropriate antisera.

Large bottles of HeLa cells can be utilized for the preparation of the three poliomyelitis, Coxsackie-B and A-9, adenovirus, and echo virus antigens, for use in complement fixation, neutralization, and hemagglutination-inhibition tests.

3. Embryonated Eggs

Embryonated eggs are used primarily in our laboratory for the isolation of influenza viruses and the preparation of influenza antigens to be used in the serological tests or for the immunization of rabbits for anti-influenza virus sera. Eleven day embryonated eggs were obtained from the local hatchery, and the usual inoculation technics were employed.

Results of the Year's Operation

There have certainly not been a sufficient number of tests made during the year to have any statistical significance. The following citations are approximations intended as an indication of what clinicians are apt to expect of a diagnostic virology department in a medium-sized hospital, and to serve as a gauge of the quantity of materials required in a small diagnostic virology laboratory.

* Microbiological Associates, Inc., Washington, D. C. Difco Laboratories, Detroit 1, Michigan.

There were approximately two hundred and thirty seven requests for the serological study of viral diseases, about equally divided between the respiratory and neurological categories. These tests revealed the expected antibody levels occasioned by the widespread poliomyelitis and Asian influenza immunizations in the area. The clinicians were quite interested in the demonstration of the few instances of antibody titer rises in cases of non-paralytic polio among the vaccinated children, as well as the titer rises in cases of Asian influenza. They had not expected the existence of quite so widespread an incidence of Coxsackie virus in the population as the tests revealed. The most satisfying experience of the year was the demonstration of the presence of St. Louis encephalitis in a patient whose symptoms were challenging considerable interest among the medical staff members.

During the recent influenza epidemic of the Asian type we had approximately fifty requests for isolations of the virus. Throat washings inoculated into embryonated eggs yielded about seventy-five per cent recoveries, which was understandably high since the doctors had voluntarily excluded from hospitalization all influenza cases except those who had complications or whose home surroundings were not conducive to good care. Two aged and debilitated patients died of fulminating pneumonia. In both instances lung tissue submitted from the autopsy room yielded Asian influenza virus. There was recovery of a Coxsackie A-9, later confirmed by the State Laboratory, from a child with "non-paralytic poliomyelitis," and a number of adenovirus specimens in cases of mild upper respiratory infection. There has been no polio virus recovered since the isolation technics were inaugurated after the "polio season."

Contributions of the Virology Laboratory

Although one doctor complained that the establishment of the virology department had robbed him of a perfectly good wastebasket for undiagnosed cases, the medical staff's appreciation of the service has been shown by their increasing use of it. Many of the doctors are pleased to have a scientific basis for their diagnosis, and the students-at-heart clinicians are enthusiastic with the virology assistance in classifying the symptoms they note in some of the undefined mild cases of respiratory and meningeal diseases. Most of them feel that their acumen in diagnosis is being enhanced and therefore their ability to help the patient is more objective. This is especially true as we are beginning to lay the pattern of the respiratory diseases in the locality at various seasons.

From the individual patient's viewpoint there is little benefit from a laboratory "test" which cannot confirm or detect the agent during the acute phase of his illness. But relapses are a different story. And often both his own and his family's psychological attitude is improved if the retrospective diagnosis reveals that the paralysis due to Coxsackie virus will eventually recede. The encouraging prognosis relieves their anxiety.

The greatest advantage of diagnostic virology lies in the services given the community in which the laboratory is situated. The prevalent

type of encephalitis and of respiratory diseases is revealed to the medical profession of the area thus enabling them to detect the first symptoms earlier and with greater certitude. Another valuable point in this period of mass immunizations is that the survey work of the local virology laboratory would indicate whether the populace should receive booster shots for the previously prevalent strains of virus, or be immunized against an agent new to the vicinity.

Summary

A diagnostic virology department has been established in a medium sized hospital away from university or medical research centers with a minimum expenditure, utilizing the services of medical technologists having previous training in microbiological and serological technics. What has been done in the virology department on a limited scale is similar to what is being done in larger laboratories. The point to be noted is that outlying laboratories should not be hesitant to establish virology departments.

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LABORATORY IDENTIFICATION AND CLASSIFICATION OF STAPHYLOCOCCI*

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Staphylococcal infections in hospitals has been the topic of several panel discussions and conferences at recent regional and national meetings.[7, 12, 15, 30, 37, 38, 45] Also, the epidemiology, therapy, and management of these infections have been the subject of many publications. Furthermore, the staphylococcal problem is now known to be not only a nosocomial but also a community problem reaching out to the private doctor's office and the small clinic in addition to larger institutions. The problem of staphylococcal infections,[20, 25, 29, 40] as well as that of staphylococcal food poisoning[14, 32] makes a knowledge of staphylococci essential for the medical technologist in bacteriology.

The purpose of this paper is to discuss the characteristics of staphylococci which are useful in its isolation and identification in the laboratory. The classification given in *Bergey's Manual of Determinative Bacteriology*, Seventh Edition, will be followed. Products such as leucocidin, fibrinolysin, and enterotoxin will not be discussed; however, their properties are reviewed elsewhere.[2, 3, 24] It has been possible to separate staphylococci into pathogenic and nonpathogenic groups by serological means,[4, 33, 34] but at present this is not a routine test. Bacteriophage typing,[5] on the other hand, has proved quite valuable for the identification of particular strains in epidemiological work. It is a tool, however, for research centers rather than routine diagnostic laboratories.

Characteristics Used In Recognition And Identification

HEMOLYSIS: Staphylococci frequently produce a zone of hemolysis around their colonies on blood agar plates, but this is true not only of those species which are pathogenic *(Staph. aureus)*, but also of those which are only parasitic *(Staph. epidermidis)*. The degree of hemolysis is dependent upon the species of erythrocytes used, the type and number of hemolysins acting on them, and the temperature, atmosphere, and other conditions of incubation.[3] Although rabbit erythrocytes are most susceptible to hemolysis by *alpha*-toxin, which is considered to be the most important pathologically, sheep erythrocytes may also be lysed and are more widely used for blood agar plates because of convenience and their superiority for detecting hemolytic streptococci. Human erythrocytes are less desirable for this purpose. They are not lysed by *alpha*-toxin, their complete hemolysis being caused by *delta*-lysin.[28] The inhibition of staphylococcal *delta*-lysin by micrococci has been reported by Liu[26] and occasionally observed here. Experience in this laboratory with both fresh human and sheep blood has clearly demonstrated the superiority of the latter for blood agar. Not only do antibacterial substances frequently occur in human blood, but the preservative solutions in transfusion bottles, in which the blood is collected, inhibits hemolysis. Because of its variability, hemolysis of blood agar is of only secondary importance in the identification of staphylococci. The terms "hemolytic" or "non-hemolytic" should not be applied to staphylococci unless the species of erythrocytes or the toxin is named. The *alpha* and *beta* toxin are not to be confused, however, with the *alpha* and *beta* hemolysis of streptococci.

* Presented before the 26th Annual Convention, ASMT, Milwaukee, Wisconsin, June, 1958.

COLONIAL MORPHOLOGY: Young surface colonies of staphylococci on blood agar are circular, opaque, raised, smooth and glistening, with a butyrous consistency and an entire margin. When they are older, they become umbonate. As autolysis of the center develops, multiple papillae are formed.[23] On gross morphology alone, micrococci are not distinguishable from staphylococci except that the latter are usually larger. *Staph. epidermidis* and *Staph. aureus* are also similar morphologically. Some diphtheroids resemble staphylococci so closely that microscopic examination is necessary to distinguish them. Streptococci and yeast occasionally cause confusion. The streptococci may be differentiated by their negative catalase reaction, and the yeasts, by microscopic examination of stained or wet preparations.

COAGULASE REACTION: The coagulase test is the most important single *in vitro* procedure for pathogenic staphylococci. The test, originally described by Loeb[27] using dilute goose plasma, is now performed with rabbit or human plasma. Studies using human plasma and rabbit plasma show little difference in the results of properly performed tests, but indicate that rabbit plasma is slightly superior to human, and the lyophilized commercial products more uniform than plasma selected at random.[6, 31, 42, 43] The tube method has been described so often that repetition is not needed. The slide test[8, 31] is a valuable screening procedure since results positive by this method have always been positive by the tube method. Comparison studies[31, 42] have shown only tests negative by the slide method need be confirmed by the tube procedure. The slide test is actually one for "clumping factor" (so-called "bound-coagulase") whereas "free-coagulase" is tested by the tube or plate method.[24] After completion of the slide test, the slide may be stained and examined microscopically to confirm the presence of staphylococci.

Evans *et al*[13] have pointed out an important source of error in coagulase testing. Enterococci are salt tolerant and may exhibit hemolysis, gelatin liquefaction, and the fermentative patterns and morphology of staphylococci. Many are citrate positive. Thus they may give a false positive coagulase tests since the citrate has been utilized by the enterococci and is not available to prevent clotting of the plasma. This does not occur, of course, if other anti-coagulants such as oxalate or heparin are used in the plasma.

Graham, Pendergast, and Henderson[18] have developed a technique combining the tests for coagulase, and *alpha*-toxin production into one procedure. The test is performed by inoculating a tube containing broth, rabbit plasma, and washed rabbit erythrocytes which is incubated in a 37° C water bath for 3 hours and observed for lysis of the cells and coagulation of the plasma.

MANNITOL REACTION: The ability to ferment mannitol and coagulate plasma separates *Staph. aureus* from *Staph. epidermidis*. Some strains may oxidize mannitol but not ferment it; however, the correlation is sufficient that reactions with mannitol and an indicator in an agar plate are frequently used. Nevertheless, either oxidation or fermentation reactions should be read after not more than 36 hours incubation.

PIGMENT: Traditionally, *Staph. aureus* (or identical organisms bearing various synonyms) is, of course, a golden staphylococcus. The pigmentation is enhanced by secondary incubation at reduced temperatures

(refrigerator or room temperature). Milk agar has been the usual substrate for pigment production, and recently Johnston[21, 22] has shown calcium to be the responsible ion, and that its effect is antagonized by magnesium. Because of this, many routine media, such as Trypticase Soy Agar or Eugonagar are adequate for detecting pigmentation. At present, pigmentation is a criterion for recognition, but is no longer one for classification, of staphylococci.

Media Used For Isolation And Identification Of Staphylococci

A good nutrient base such as Trypticase Soy Agar (plus 5% defibrinated sterile sheep blood, for the reasons previously mentioned) is an excellent medium and widely used. The fact that organisms other than *Staph. aureus* will grow is either an advantage or a disadvantage depending upon the purpose of the study and the specimens to be cultured. Since some workers wish to isolate *Staph. aureus* only, selective media are employed.

Since staphylococci tolerate high concentrations of salt,[9] Mannitol Salt Agar is selective and differential because mannitol and phenol red are added to a base containing 7.5% (*w/v*) sodium chloride. *Staph. aureus* appears as a yellow colony surrounded by a yellow zone, whereas *Staph. epidermidis* appears as a small colony in a red or purple zone.

Staphylococcus Medium # 110[10] and its modification, Chapman Stone Agar,[11] are also high salt content media which contain gelatin as well as mannitol. Since gelatin liquefaction is no longer considered as a differential test in classification, these media are now used less frequently. Pigment production, however, is quite good on them.

Tellurite Glycine Agar[47] is a medium containing lithium chloride, potassium tellurite and glycine as selective agents. Coagulase positive staphylococci form black colonies whereas most other organisms fail to produce visible growth in 24 hours. Coagulase negative staphylococci are completely inhibited or form grey colonies.

Classification Of Staphylococci

Before one attempts the use of scientific names for organisms isolated in the laboratory, it is important that something of the philosophy of taxonomy be understood. First, there should be a realization that all such classifications and "labelings" are artificial. The categories are man-made and unknown to the organisms concerned. Second, the taxa have been created for convenience. They are descriptive abbreviations. Variation occurs in all forms of life. The proper attitude, then, is one of selecting and using a name which most closely summarizes the characteristics exhibited by an organism, rather than trying to fit an organism with a particular name, for taxa are merely concepts, albeit useful ones, and not definite entities.

Considerable controversy exists concerning the taxonomic status of micrococci and staphylococci. These arguments have been recently reviewed by Thatcher and Simon.[41] It is generally agreed that the coagulase positive staphylococci represent a homogeneous group. They are designated as *Staphylococcus aureus* because of priority although many prefer the term *Staphylococcus pyogenes*. They differ from coagulase negative staphylococci in a number of characteristics such as bacteriophage susceptibility,[5] phosphatase activity,[46] tellurite reduction and

glycine inhibition,[47] viability in leukocytes,[16] deoxyribonuclease activity,[44] and neomycin susceptibility.[17] This last characteristic is now being used in this laboratory as a basis for a new selective and differential medium for *Staph. aureus.*

The coagulase positive, mannitol fermenting staphylococci are *Staph. aureus,* and coagulase negative, mannitol negative staphylococci are designated as *Staph. epidermidis.* Both of these reduce nitrates to nitrites. In routine work, then, colonies presumed to be *Staph. aureus* may be tested for coagulase production. If this test is negative, and the organism is seen by stain to be a staphylococcus, a positive test for the reduction of nitrates to nitrites will designate the organism as *Staph. epidermidis.* If nitrates are not reduced to nitrites, the organism is classed with the micrococci. The term *Staph. albus* is often without meaning. Sometimes it denotes a coagulase positive white variant of *Staph. aureus,* but frequently it means what is now meant by *Staph. epidermidis.* The same is true of *Staph. citreus* except that it is pigmented.

In addition to the tests for coagulase and *alpha*-hemolysin production, the antibiotic susceptibility pattern, or antibiogram, is often helpful. Certain strains have characteristic antibiotic profiles. Although antibiotic resistance *per se* is not indicative of pathogenicity, it has some epidemiological value.[35] Furthermore, unusual patterns such as resistance to many antibiotics, suggest the possibility of diphtheroids, yeasts, or other contaminants, especially if the organism appears resistant to bacitracin or neomycin, since staphylococci are almost invariably sensitive to these antibiotics.

Phage typing is, of course, the final step in the labeling of pathogenic staphylococci. It is a test for the susceptibility of bacteria to virus infection. It can show two strains to be different, or that they may be identical. It cannot prove two strains to be from identical sources. Phage results may be obtained from various typing centers, but cultures should not be sent without performing all the previously mentioned tests and forwarding the results, together with other pertinent information, along with the culture.

Summary

1. The current prevalence of nosocomial infections and the importance of the staphylococcal problem make the rapid and accurate identification of pathogenic staphylococci a necessity.

2. The important characteristics for laboratory diagnosis such as production of coagulase, *alpha*-hemolysin, and mannitol fermentation are given. Media for staphylococci are described.

3. Factors to be considered in the application and interpretation of these tests are discussed.

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ABSTRACTS (continued)

URINARY 17-KETOSTEROID EXCRETION, BLOOD EOSINOPHIL LEVELS AND ADRENOCORTICAL FUNCTION IN ADULT MALE EGYPTIANS

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Eighteen healthy male Egyptians (mean age of 32 years) had a mean urinary total of neutral 17-Ketosteroid output of 8.9± 1.20 (range 5.2-14.9) mgs. in 24 hours. This is lower than that reported for Western subjects but not lower than that reported for non-Western males. Mean circulating eosinophil level was 230± 49.4 cells per c.mm. blood.

After intramuscular ACTH the 17-ketosteroid excretion rose from a mean value of 8.6± 1.30 mgs. per 24 hours to 13.1± 1.88 mgs. per 24 hours and the mean eosinophil level in these males fell from 224± 58.5 per c.mm. blood to 102± 28.1 per c.mm. blood during the latter half of treatment; 4 to 6 days after onset of the ACTH the 17 ketosteroids level was 9.5± 1.70 mgs. per 24 hours and the eosinophil count was 189± 55.2 cells per c.mm. blood. These indices to ACTH indicate normal adrenocortical function.

THE HOSPITAL LABORATORY AS A BUSINESS ENTERPRISE†

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If a business man wants to stay in business he must furnish a sound product or a useful service at a price that people can pay. In order to do this he must control the quality of his product or service and he must maintain the efficiency of his organization so that he neither goes broke nor prices himself out of the market. To keep his customers he must process orders, or requests for service, promptly and accurately. If we are to give patients good laboratory service at a reasonable price we must operate the hospital laboratory in a similar business-like manner.

COMMUNICATION

Good service begins with prompt and accurate processing of test requisitions and prompt reporting of test results. Our approach to the solution of the problem of good communication has been based upon the development of a system which permits us to pin-point the responsibility for delayed or erroneous tests. Each department of the laboratory has a separate, color coded form. As requisition forms for use at nursing stations, etc., these are put up in books or pads with carbon papers. As report forms for the laboratory they are made up in "snap apart" sets of two or three copies with built-in, one time carbons. When a test requisition is made out and sent to the laboratory a carbon copy stays in the requisition book at the nursing station. The original copy, which the nurse has dated and initialed, is brought to the laboratory and put through the time stamp machine. When a doctor complains that we have missed a test he ordered we look in the requisition book on the floor. If there is no carbon copy, we have evidence that no requisitions were filled out. The time stamp fixes the time that the requisition was received. The old recurring arguments about who is responsible for delayed or missed tests have almost completely dissappeared.

The requisition form which has been filled out by the nurse and time stamped is turned over to the technician who collects the specimen and processes it. The results of the tests are written directly on the slip which is returned to the typist. The result is then copied on a report slip which is an exact duplicate of the original requisition, thus minimizing errors such as typing a result on the wrong line.

After the report has been typed, the original typed copy is placed on the patient's chart by laboratory personnel. The carbon copy goes to the attending physician for his office records. The original requisition with a complete record of the transaction, including the initials of the nurse who filled out the slip, the initials of the technician who performed the test, the time stamped record of the receipt of the requisition, plus any calculations which may have been made on the back of the slip, then goes into our permanent file.

Even with this system problems still arise.

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† Received for publication August, 1958.

1. Wrong Orders:

Every laboratory should have a detailed procedure book for the nursing personnel with instructions for filling out requisitions, patient preparation, etc. This should minimize ordering errors. If, however, you feel that the wrong test has been ordered, talk to your pathologist or the attending physician. If neither is available, run the test as ordered. There is a saying in the printing profession, "Follow the copy even if it blows out the window." The requisition slip is your justification.

2. Verbal Orders:

Confusion sometimes results from misunderstood verbal orders. Written requisitions should be required on all tests. If this is not possible, (for instance when the doctor calls in by telephone) write down his request and read it back for confirmation.

3. Stat Orders:

All stat requests should carry an extra charge, not as a punitive measure, but because it costs a lot more to do stat tests which require upsetting the laboratory routine. Most laboratories must employ at least one extra technician in order to provide stat and night emergency service. It is logical that the patients who benefit from this extra service should help pay its cost. The extra charge discourages "stat" requests that are not really emergencies. Of course, all tests should be processed as promptly as possible.

4. Confusion in Handling:

Confusion in handling, typing, and sorting of slips will be minimized by a systematic flow of slips to the various laboratory departments and from the departments to the typist. A sorting rack is helpful. A billing slip, filled out at the time the requisition is received, and sent to the business office when the tests are completed also serves as a check on work in progress.

ACCURACY

The second factor in good service is accuracy of test results. Since the patient cannot check our results, and even the physician is forced to take our word for the accuracy of most tests, we are under great obligation to check our own results. A number of surveys have shown that errors occur very commonly in hospital laboratories and frequently are of such proportion as to render the reports worthless and even dangerously misleading. These surveys include the investigation of hemoglobin tests by Karr and Clark[1] and the surveys of chemical tests by Belk and Sunderman,[2] Marsters,[3] Snavely and Golden,[4] and Wooten and King,[5] all of which showed wide and alarming variations in results. On the basis of this unsatisfactory showing, public health agencies are becoming concerned about the problem and we must do something about it whether we want to or not. Some methods of quality control are available, more need to be developed.

Sources of Error

If we are to evaluate our test procedures critically and attempt to detect and eliminate errors we must be thoroughly familiar with the sources of error. Some of these lie outside the clinical laboratory and are beyond the control of the technologist. The analyst, however, should be aware of the influence of these factors on his final result so that he can warn the attending physician that the results may be misleading.

The first source of error is the physiological variation which may take place in the patient due to his reaction to the operation required to collect the specimen. An example is the elevated total white count on an agitated child.

Another source of error is the technique used in collecting the specimen. For example, the cup full of saliva which is submitted as a sputum specimen may result in a falsely negative report on a patient with active tuberculosis.

We are all aware that some specimens are perishable and examinations cannot be delayed, and others must be preserved in special ways. Nevertheless, there is sometimes pressure to go ahead and attempt to use an unsatisfactory specimen to save the trouble of collecting another.

The necessity of constant identification of every specimen and every report should be mentioned. It does no good to do a technically perfect procedure and report it on the chart of John Smith when the specimen came from William Jones. If the test was a cross match, you may kill Mr. Smith.

It is obvious that if we are to do accurate quantitative work the glassware we use for measurement must be accurately calibrated, either at the factory or in our own laboratory. Riopel[6] has emphasized the potential errors in the use of precalibrated colorimeters and spectrophotometers. Any calibration applies only to the actual reagents and technique, and then should be rechecked constantly.

The consistent accuracy of results produced in any laboratory will depend a good deal on the selection, training, and supervision of the technologists and upon their ability and experience. An excessive work load, with resulting fatigue and discouraging back log of work, is bound to have a deleterious effect upon the quality of work. The distractions of frequent interruptions, of excessive noise, and of undue and unwarranted criticism all diminish efficiency and destroy accuracy.

The fact that a method has been carefully worked out, and the measuring instruments and glassware meticulously calibrated does not insure that this method will be forever accurate and reliable. The contamination or decomposition of reagents, the evaporation of solvents, as well as undetected variation in techniques may eventually lead to totally unreliable results.

"Quality Control"

Since there are so many factors which influence the accuracy of the clinical laboratory the practical control of accuracy must depend upon our ability to detect errors. Industry has long used various methods of spot checking products to insure that the finished articles meet certain specifications. This method of spot checking is called "quality control."

This term has been borrowed and applied to various techniques used in the clinical laboratory to evaluate the accuracy of tests.

In clinical chemistry, quality control, at least in theory, is relatively simple: Include a standard solution with each group of tests. Since a pure solution is not identical with serum, plasma, or blood, an even better check may be obtained by using serum standards processed from out-dated, or rejected bank blood, or one of the commercially available synthetic sera. Quality control charts and graphs made in various ways have been shown to be helpful in evaluating the work of the department.[7, 8, 9] We have found that simple "scatter" graphs showing the distribution of results each day can reveal errors, and indicate methods which should be investigated. Statistical control and evaluation of various procedures can be revealing and serve as a valuable check on technique.

In hematology, the problem of quality control is more difficult because we do not have, except in the case of hemoglobin, readily available standards. Statistical control of red counts as suggested by Lancaster[10] and Elsdon-Dew[11] gives a good idea of the quality of work being done, but are very cumbersome and require a considerable knowledge of mathematics. Statistical or graphic analyses of mean corpuscular constants offers a distinct advantage in the evaluation of the work of the hematology department. We have used both methods and find that graphic evaluation is more dramatic, but calculating the standard deviation of a series of mean corpuscular hemoglobin values from patients with 4,000,000 or more red cells is more readily accomplished. The mean value for such a series should be between 29.0 and 29.5 micro micrograms. Plus and minus two standard deviations should not exceed the range 27 to 32. Deviations from these values suggest errors which should be investigated. Calculation of standard deviation by the method explained in Sunderman and Boerner[12] takes only a few minutes.

The control of white cell counting depends in part on the evaluation of the stained smear for the differential count. This will detect gross errors and probably will be a sufficiently good check for ordinary clinical work. The statistical control suggested by Lancaster[10] and Elsdon-Dew[11] is also applicable to white cell counting, but, again, is cumbersome.

The new methods of radioactive iodine uptake and protein bound iodine give accurate checks on the results of the BMR test and can be used in situations where the BMR is not applicable. Unfortunately, both require expensive equipment and special training. A simple quick check on the accuracy of a given BMR can be obtained by taking the patient's blood pressure and pulse rate at the time of the test. The formula: pulse pressure + pulse rate —111 = BMR, gives a fairly accurate check.

How many of you have checked the calibration of the urinometer which you use? Scatter graphs of the daily urine specific gravity readings may bring consistent errors to your attention. For example, if all values fall between 1.014 and 1.040 something is wrong.

A simple review of the urine pH readings day after day may show that most of the specimens are strongly alkaline and suggest that they are being collected the evening before and are sitting overnight in a warm room. Obviously the findings in such urines are far from reliable.

In some phases of laboratory work quality control has long been a part of our routine methods. This is true in serology where positive and negative controls are routinely included in most tests, and in prothrombin testing, where the comparison of each day's unknowns with a "normal" control acts as a reasonably good check upon the accuracy of the procedure.

Finally, in blood banking quality control methods are probably used more extensively than in any other field, since the results of an error here can be so disasterous. The back typing of blood by checking the serum against known cells, and the use of macromolecular solutions and anti-globulin tests, all serve as effective quality control measures.

The final proof of accuracy of laboratory procedures is in their correlation with the clinical evaluation of the patient and their final correlation with the findings at surgery or at autopsy. The price of accuracy, like the price of liberty, is eternal vigilance. The reward is the confidence of the hospital staff and the welfare of the patient.

SAFETY MEASURES

Fire:

Many of our reagents and solvents are inflammable or actually explosive. Are you careful that the ether, benzene, etc., are not stored or used near bunsen burners, the flame photometer, open switches, hot lamps, hot plates, etc.? Do you have the right type of fire extinguishers readily available? Do you know how to operate them? Have they been checked for proper filling and operation in the past year? In the past five years?

Poisonous, caustic and irritant chemicals:

Are all containers adequately labeled and possible hazards indicated? Are the outside surfaces of containers kept clean? Do labels caution against pipetting of poisonous substances?

Irritating and Toxic Fumes:

Are adequate fume hoods provided and used? Is the laboratory adequately ventilated?

LABORATORY ORGANIZATION

Delegation of Authority and Responsibility:

In a small laboratory with only a few workers, a very simple staff setup is usually adequate. In larger laboratories with many workers in different categories and with different degrees of training, some formal chain of command with delegation of authority and responsibility helps to avoid or solve many problems. In a laboratory with 15 or more workers this organization might take the following form:

1. Chief technologist
2. Department supervisors (registered technologists with some experience)
3. General technologists
4. Aides
5. Secretaries and clerical workers
6. Maintenance personnel

The chief technologist would be responsible for the over-all operation of the laboratory and school of technology and for general planning and assignments. The department supervisors would be responsible for all work done in their own departments by general technologists, aides, etc. and would assist in training students and aides. These added responsibilities should carry extra salary benefits.

Job Descriptions

Every worker in the laboratory should know what is expected of him. Job descriptions help to accomplish this. It should be emphasized that these job descriptions are positive, not negative; that is, they define major areas of responsibility, but do not exclude other work and should not be used as a refuge and excuse to avoid other assignments where these are necessary.

Even in small laboratories arrangements must be made for personnel time off, for vacations, and for sick time. For this reason it is essential that at least two people in the laboratory can do each test.

EFFICIENCY

While wasted time is the most expensive inefficiency in the clinical laboratory, the inefficient handling of reagents and supplies can also be the source of excessive costs.

Personnel

How many workers are needed in your laboratory? Several factors enter into the problem of determining personnel requirements.

a. Number of tests to be done. This seems simple enough, but is complicated by the fact that there is no uniformity in the methods of counting tests.

b. The time it takes to do each test.

c. The time needed for other work in the laboratory, including reagent preparation, clean up, typing, filing, etc.

Several standards for efficiency have been published. Several years ago the U. S. Public Health Service stated that one technologist should do between 3,000 and 7,000 tests per year. This gives wide latitude and does not define how tests shall be counted, nor does it consider the variation in time between simple and complex procedures. Starkey[13] recently suggested that each procedure in the clinical laboratory be assigned work units, the number depending upon the complexity of the test and the time required for its performance. On the basis of such work units, he states that each technologist working in a small laboratory should perform from 1,600 to 1,700 units per month. More recently, Arthur Allen[14] and Steinle[15] have published articles on time per test studies. Results obtained in such studies will depend upon whether only the actual test procedure is timed or whether all of the time the technician spends in the department is included, taking into account reagent preparation and other activities. Simple personnel time record sheets in each department on which every worker signs in and out can be summarized weekly or monthly to give a reasonably accurate picture of time per test, and may reveal inefficiency.

Supplies

If supplies are ordered too frequently and in small batches you will pay higher prices for the small lots, shipping costs will be higher, and you are likely to run into frequent shortages and emergency phone orders. On the other hand if excessive quantities are ordered at one time, some products will spoil, biologicals may be outdated, and there are likely to be supplies left when methods are abandoned. In addition, large quantities require excessive storage space.

Where the monthly volume of supplies ordered and received is fairly high a stockroom organization is worthwhile. This can be handled by a clerical worker, freeing the technologists for professional tasks. If a storeroom is not available this can be set up in available drawer space. All supplies are checked into the storeroom and recorded on perpetual inventory cards. Each department requisitions reagents and supplies as needed, preferably on a weekly basis. The requisition slips are kept and totaled each month for the business office. Supplies dispensed are noted on the cards.

This provides visual evidence of:

1. Supplies on hand.
2. Rate at which supplies are being used.
3. Time when new stock should be ordered and how much.

This gives the hospital business office a much more valid picture of the monthly supply costs of the laboratory.

From personnel time records and departmental requisitions, it is possible to estimate the actual cost of doing various tests. This permits determining laboratory charges on a more realistic basis than the traditional one where hospital A charges $5.00 for a C.B.C. because hospital B and C charge $5.00.

Efficiency implies the best possible use of time and equipment consistent with good results. For example: Microchemistry saves time, reagents, equipment, blood and space. It is just as accurate and no more difficult than the usual macro methods. The use of quadrant plates in bacteriology saves glassware, incubator space, handling, and sterilizer space. Clean-up time and sterilizing equipment can be conserved by using disposable lancets, needles, specimen containers, petrie dishes, etc.

Proper arrangement of equipment and supplies at each work area so that a whole series of tests can be performed without the worker getting up, saves both time and energy.

Ideally, everyone should work at the maximum of his capabilities. We can't afford to have technologists do work which an aide or secretary or janitor can do as well. We can't afford to have people do work that can be done by machines. Lest you be concerned that machines will take over your jobs, remember that in industry the increased income of the worker is due primarily to better tools and resulting increased productivity. Without this, increased pay would simply have priced products out of the market. It is wasteful to use human labor for a job which a machine can do as well or better. There are still plenty of things which require judgment and can be done only by trained human workers.

Talk isn't cheap, not if it's idle gossip. You can't do efficient and accurate work and chatter at the same time. Is overtime the price you pay for idle talk?

PUBLIC RELATIONS

The laboratory department can function efficiently only when it has an amiable working agreement with the members of the medical staff, the nursing staff, and other hospital departments. A running battle with any or all of these can consume a tremendous amount of time and energy better devoted to productive work.

The best way to insure reasonable treatment by others is to be reasonable yourself.

Points to Remember:

1. Every procedure you do is subject to error. A request to repeat the test is not an insult, nor does it impugn your technical ability. Repeat the test without charge. Best preventive—repeat any test with questionable results *before* you report it.
2. Most of the people you deal with are under tension. Be diplomatic and considerate. The sick patient and the doctor who is worried about him may be upset by some remark or attitude of yours which would not bother them under normal circumstances.
3. One of the surest ways to create resentment is to demand that something be done in a certain way "because I say so!" Explain your request, preferably before you make it.
4. If you need something for the laboratory (a new instrument, a new operational procedure, etc.), a reasonable and convincing explanation of what it will do, why you need it, what it will cost, and what it will contribute financially or otherwise will probably get it for you. If you can't justify it, you probably don't need it.
5. Encourage suggestions from the doctors, nurses, the business office, etc., on ways to improve service. Get everyone on your team. If a problem comes up, review your procedures to see if they need revision to prevent recurrence of the problem.

It has been said that the test of a good administrator is not whether he has problems, but whether he has the same problems he had last year.

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MEDICAL TECHNOLOGISTS WANTED

Wanted: Medical Technologist, ASCP, Male. 40 hour week, liberal personnel policies. Full time pathologist in charge of laboratory. Salary open. Apply: Pathologist, Winter Haven Hospital, Inc., Winter Haven, Florida.

Wanted: Qualified Medical Technologist. New 500 bed air-conditioned hospital. Salary depending on experience. No call. Write to: Sister Rose Marie, Administrator, St. Vincent Hospital, Green Bay, Wisconsin.

Wanted: Male or Female. Laboratory Technologist (ASCP or eligible) for a 65 bed community hospital in the "Beautiful Berkshires," a year-round vacation and sports area. $350.00 to $450.00 per month depending on training, experience, and degrees, liberal personnel policies. Dept. is under supervision of a Board Certified Pathologist. Apply: Administrator, W. B. Plunkett Memorial Hospital, Adams, Massachusetts.

Wanted: Medical Technologist capable of performing all general laboratory tests including PBI Determinations. Salary commensurate with training and experience. Apply: Administrator Andrew Kaul, Memorial Hospital, St. Marys, Pennsylvania.

Wanted: Medical Technologist, ASCP or eligible, for small general hospital. Apply Superintendent, Aston Park Hospital, Asheville, N. C.

WANTED: MT (ASCP)

for 38 bed hospital and 10 doctor clinic. Salary—commensurate with training and experience. Good personnel policies. Three other technologists in laboratory. Small town 60 miles from Houston, Texas. Write

C. H. RUGELEY
Business Manager
Box 548
Wharton, Texas

Wanted: Medical technologist (ASCP) fo Loveland Memorial Hospital, Lovelan Colorado. Salary open. Telephone Nor mandy 7-0780. Write or call.

Clinical Laboratory Technologist, ASCP o eligible. Liberal vacation, sick leave, n call. Salary depends upon qualification 400 bed hospital. Contact Personnel Offic Iowa Methodist Hospital, Des Moines Iowa.

(Continued on Page XXX

AMENDMENTS TO BY-LAWS
AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Adopted June 24, 1957

BY LAWS

ARTICLE IV
Membership

Section 1. The membership of this Society shall consist of the following classes:

(a) Active Member. Any person who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in microbiology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a certificate in chemistry or microbiology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, and is in good standing with that Registry, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more semester credit hours from an accredited college of which at least twenty-seven hours are in science including chemistry and/or biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.

(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing or special committee of this Society.

(c) Student Member. Any person enrolled in an approved School of Medical Technology or in an under graduate program which is pre-requisite to enrollment in an approved school is eligible for Student Membership in this Society. He may enjoy this membership until he becomes a registered M. T. (ASCP) by the Board of Registry of Medical Technologists of ASCP or until he becomes ineligible to take the examination for such registration. Upon successful completion of the Registry examination he shall automatically be billed for Active Membership in this Society. A student member is entitled to all the privileges of membership in this Society except voting, holding office, and serving on any Board or standing or special committee of this Society.

(d) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendations for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing or special committee of

this Society. He shall be exempt from payment of annual dues.

(e) Corresponding Member. Any person who is resident of, and employed as a medical technologist in, a foreign country, who is in good ethical standing, and who is a member of a government—and/or medical association—recognized association of medical technologists in that country, shall be eligible for Corresponding Membership in this Society. He shall make application through the association of which he is a member. A corresponding member shall enjoy all the privileges of this Society except voting, holding office, serving on any Board or standing or special committee of this Society or any of its activities from which he might be excluded by laws governing aliens. A corresponding member shall forfeit all privileges of membership upon changing his place of residence or employment to a geographical area where there is a constituent society of this Society.

Section 2. Application for active or affiliate membership in this Society shall be made on a form prescribed by this Society. The transmittal to the executive secretary of a list of active and affiliate members in good standing in a constituent society, together with their annual dues shall establish them as members of this Society.

Section 3. Application for student membership in this Society shall be made on a form prescribed by this Society. The transmittal of this application to the executive secretary of this Society together with the required amount of dues shall establish him as a student member of this Society.

Section 4. Any member of this Society whose conduct is detrimental to this Society may be expelled by a two-thirds vote of the House of Delegates provided he has been given thirty days notice, signed by a majority of the members of the Board of Directors and sent by registered mail, stating the charges filed against him and informing him of the date of the meeting when his expulsion will be voted upon. The member so charged shall have the privilege of appearing before the House of Delegates and he may present his defense in person or through other active members of the Society of his selection.

Section 5. Any person who has been expelled from membership may be reinstated upon adoption by a two-thirds vote of the House of Delegates of his application for membership, provided the same is accompanied by annual dues to this Society plus a reinstatement fee of five dollars ($5.00).

ARTICLE V

Code of Ethics

Being fully cognizant of my responsibilities in the practice of Medical Technology, I affirm my willingness to discharge my duties with accuracy, thoughtfulness, and care.

Realizing that the knowledge obtained concerning patients in the course of my work must be treated as confidential I hold inviolate the confidence (trust) placed in me by patient and physicians.

Recognizing that my integrity and that of my profession must be pledged to the absolute reliability of my work, I will conduct myself at all times in a manner appropriate to the dignity of my profession.

ARTICLE VI

Meetings

Section 1. The Society shall hold an Annual Session which shall include one or more general or scientific meetings of the members and not less than two meetings of the House of Delegates at least two of which shall be held on different days. The Annual Session shall be held at the time and place determined by the House of Delegates at any preceding Annual Session. Emergency changes concerning the time and place of an Annual Session may be made by the Board of Directors.

Section 2. The Board of Directors shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within the two weeks prior to the first meeting of the House of Delegates at each Annual Session.

Section 3. The Advisory Council shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary

business and shall hold a meeting within two weeks prior to the first meeting of the House of Delegates of each Annual Session.

ARTICLE VII

Finances

Section 1. The annual dues for active and affiliate membership in this Society shall be eight dollars ($8.00) per annum.

Section 2. (a) Dues for student membership in this Society shall be $3.00 per annum. (b) Any dues paid by a student member who becomes eligible and applies for active membership prior to January 1 will be refunded on the basis of $.25 per month for any overlapping period retroactive to July 1.

Section 3. The dues for corresponding membership in this society shall be four dollars ($4.00) per annum.

Section 4. (a) Dues to this Society shall be payable to the treasurer of the constituent Society of the geographical area in which the member is employed, resides, or maintains his status as a student, or in the event no such constituent society exists, to the executive secretary of this Society.
(b) Annual dues for active, affiliate and corresponding members to this Society are payable on or before July 1 of each fiscal year.
(c) Dues for a student member may be renewed at the end of 12 months for another year, provided he has not in the interim become eligible for active membership in this Society.

Section 5. (a) Any active, affiliate, or corresponding member who cannot pay dues for the current fiscal year shall notify this Society by sending written notification direct to the executive secretary not later than September 1st of that year.
(b) Any active, affiliate, or corresponding member who fails to pay annual dues to this Society by September 1st shall have his name removed from the membership list.
(c) A person whose name has been removed from the membership list of this Society may be reinstated at any time provided he meets the requirements for membership of both his constituent society and this Society. Unless a person has previously resigned, to be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current year's dues to this Society plus a reinstatement fee of two dollars to this Society.
(d) Any active or affiliate member who changes his residence or place of employment to another state, territory or nation shall become a member of the constituent society of his new residence or place of employment for the remainder of the fiscal year without further payment of dues.

Section 6. A new applicant elected to active, affiliate, or corresponding membership in this Society after January 1st and prior to May 1st shall pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 7. An applicant elected to active, affiliate, or corresponding membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

ARTICLE VIII

Officers

Section 1. The officers of this Society shall be president, president-elect, recording secretary and treasurer.
(a) The president shall be the chief executive of this Society and shall preside at all meetings of the Board of Directors and of the House of Delegates. He shall be an exofficio member of all standing and special committees except the Nominations Committee. He shall set up such standing committees as are provided for in the By-Laws except the Nominations Committee. He shall appoint with the approval of the Board of Directors, such special committees as are needed.

Section 2. The executive secretary shall contract for technical exhibits for the Annual Session of the Society and, working with the local chairman of exhibits, shall arrange for all technical exhibits. All receipts from technical exhibits shall go into the treasury of this Society.

Section 3. The Executive Secretary shall be included as a non-voting member of the Board of Directors and of the Advisory Council.

Section 4. The Executive Secretary shall distribute to the president, the president-elect and the other officers, and to the committee chairmen and to Advisory Council members, the names and addresses of the officers and committee chairmen of constituent societies as soon after October 1 as their membership status in this Society can be determined, and such lists prepared.

Substitute: pages 4, 5, and 6 for pages 4, 5, and 6 now in your copy—substitute sections above as noted on top of page 9, ARTICLE IX. Cut apart on dotted lines.

BYLAWS

AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

Revised 1953
Amended 1954-1958

BYLAWS
OF THE
AMERICAN SOCIETY OF MEDICAL TECHNOLOGISTS

ARTICLE I
Name

The name of this corporation is American Society of Medical Technologists.

ARTICLE II
Purposes

The purposes of this Society are to promote higher standards in clinical laboratory methods and research; to elevate the status of those specializing in medical laboratory technique; to create mutual understanding between the medical technologist and physicians and all others who are employed in the interest of individual or public health; to issue charters to constituent societies; to promote the mutual aid and benefits of its members; in general to do anything and everything necessary and proper to the conduct of a society of this nature, and for the purpose of attaining or furthering any of its objects to do any and all other acts and things, and to exercise any and all other powers which now or hereafter may be authorized by law.

ARTICLE III
Constituent and Branch Societies

Section 1. This Society shall be composed of constituent societies and of such branch societies as shall be organized and sponsored by the constituent societies.

Section 2. There may be one, and only one, constituent society in each geographically defined state, District of Columbia, territory, possession or dependency of the United States and in each foreign nation. Any number of branch societies may be organized by a constituent society within the boundary of its geographical area.

Section 3. The name of each constituent society shall contain the geographical location thereof and the words "medical technologists," or the equivalent of these words. A constituent society may use the name of division of the American Society of Medical Technologists.

Section 4. A constituent society can be organized by three or more persons eligible for active membership in this Society. These persons shall petition for permission to organize a constituent society on a form supplied by the executive secretary which shall be submitted to the chairman of the Board of Directors and be approved by that Board. The petition shall be accompanied by an application fee of $5.00. When the petition has been approved, six copies of their proposed constitution and bylaws or, if the group wishes to become incorporated, six copies of their proposed articles of incorporation and bylaws shall be submitted to the constitution and bylaws committee of this Society accompanied by a list of officers and charter members. The committee shall bring a report and a recommendation concerning the chartering of this group to the House of Delegates at the next Annual Session of the Society.

Section 5. A charter for the organization of a constituent society may be granted only by the House of Delegates.

Section 6. The duties and functions of a constituent society are defined as follows:

(a) It shall send to the executive secretary of this Society the names and addresses of its officers not more than two weeks after their election, and the names and addresses of its committee chairmen not more than two weeks after their appointment.

(b) It shall send dues to the executive secretary within two weeks after members have paid, together with a duplicate, typewritten, classified list of such members with their addresses and registry numbers or degree statuses.

(c) It shall provide for the types and qualifications for membership except that it may grant active membership only to persons who meet the requirements for active membership in this Society, that only active members shall have the privilege of voting and holding office in the society; and all members of a constituent society shall abide by the code of ethics of this Society.

(d) It shall sustain its status with this Society by maintaining a constitution and bylaws in harmony with the Bylaws of this Society and by maintaining adequate supervision over the branch societies which have been organized under its direction to insure that their constitutions and bylaws are kept in harmony with the Bylaws of this Society. The types of membership and qualifications for membership in a branch society shall be determined by the constituent society under which it is organized, except that all members of a branch society must abide by the Code of Ethics of this Society.

(e) It shall submit to the chairman of the constitution and bylaws committee of this Society twelve copies of all proposed amendments to the constitution and bylaws of the constituent society at least sixty days before the approval of such committee is desired. These amendments should be submitted in context. When the proposed amendments have been approved they may be adopted. A constituent society shall report the adoption of any amendments to its constitution and bylaws to the chairman of the constitution and bylaws committee and must submit a copy of its amended constitution and bylaws for filing in the executive office of this Society within two weeks after their adoption.

(f) It shall send annually before September 15th to the executive secretary a charter fee of $1.00 which provides for the renewal of its charter.

Section 7. This Society shall be empowered to revoke the charter of a constituent society by a two-thirds majority vote of the House of Delegates provided the Board of Directors by a two-thirds vote favors the revocation of such charter and provided, further, that such constituent society shall have had at least thirty days notice of the charges filed against it and shall have had an opportunity to be heard and to defend itself before the House of Delegates. The charter of a constituent society may be revoked for any of the following causes:

(a) Failure of such society to require that all its members abide by the Code of Ethics of this Society.

(b) Adoption by the constituent society of policies or procedures contrary to the purposes of this Society.

(c) Failure of the constituent society to cooperate reasonably with this Society.

Section 8. Any constituent society whose charter has been revoked may be reinstated by a two-thirds vote of the House of Delegates upon application, provided such society is no longer in default and pays all expenses incurred, plus a reinstatement fee of five dollars ($5.00).

Section 9. Any constituent society shall revoke the charter of a branch society organized under its direction for failure of the branch society to comply with both the Code of Ethics of this Society and the bylaws under which the branch society was organized and amendments thereto.

ARTICLE IV
Membership

Section 1. The membership of this Society shall consist of the following classes:

(a) Active Member. Any person who (1) is registered M.T. (ASCP) by the Registry of Medical Technologists of the American Society of Clinical Pathologists and is in good standing with that Registry, or (2) possesses a master's or higher degree in microbiology, biochemistry, hematology, histology, parasitology, or serology from an accredited college, or (3) holds a certificate in chemistry or microbiology from the Registry of Medical Technologists of the American Society of Clinical Pathologists, and is in good standing with that Registry, or (4) holds a limited certificate in histologic technique from the above mentioned Registry and has ninety or more

semester credit hours from an accredited college, of which at least twenty-seven hours are in science including chemistry and/or biology, with lecture and laboratory in each, shall be eligible for active membership in this Society provided, further, that he is an active member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An active member shall have all the rights and privileges of this Society.

(b) Affiliate Member. Any person who holds a certificate from and is in good standing with the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists without meeting the requirements for active membership in this Society shall be eligible for affiliate membership in this Society provided that he is a member of the constituent society of the geographical area in which he is employed or resides if there be a constituent society in such area. An affiliate member is entitled to all the privileges of membership in this Society with the exception of voting, holding office, and serving on any Board or standing or special committee of this Society.

(c) Student Member. Any person enrolled in an approved School of Medical Technology or in an undergraduate program which is pre-requisite to enrollment in an approved school is eligible for student membership in this Society. He may enjoy this membership until he becomes a registered M. T. (ASCP) by the Board of Registry of Medical Technologists of ASCP or until he becomes ineligible to take the examination for such registration. Upon successful completion of the Registry examination he shall automatically be billed for active membership in this Society. A student member is entitled to all the privileges of membership in this Society except voting, holding office, and serving on any Board or standing or special committee of this Society.

(d) Honorary Member. Any person who has made an outstanding contribution to the field of laboratory medicine by his research or by his service to the profession of medical technology may be recommended for honorary membership in this Society. Recommendations for honorary membership may be made by a constituent society or by the Board of Directors. A recommendation accompanied by qualifications shall be sent to the recording secretary of this Society not less than thirty days before the Annual Session. Honorary members shall be elected by a two-thirds vote of the House of Delegates. They shall be elected every third year beginning in 1954. The year when honorary members are elected, each constituent society and also the Board of Directors shall be entitled to present the name of only one candidate for this honor. Honorary members shall not exceed in number one per cent of the active members of the Society in good standing at the time of their election to honorary membership. An honorary member shall be informed of his election by the recording secretary who shall send him a certificate of honorary membership signed by the president and the recording secretary. He shall enjoy all the privileges of membership except voting, holding office and serving on any Board or standing or special committee of this Society. He shall be exempt from payment of annual dues.

(e) Corresponding Member. Any person who is resident of, and employed as a medical technologist in, a foreign country, who is in good ethical standing, and who is a member of a government-and/or medical association-recognized association of medical technologists in that country, shall be eligible for corresponding membership in this Society. He shall make application through the association of which he is a member. A corresponding member shall enjoy all the privileges of this Society except voting, holding office, serving on any Board or standing or special committee of this Society or any of its activities from which he might be excluded by laws governing aliens. A corresponding member shall forfeit all privileges of membership upon changing his place of residence or employment to a geographical area where there is a constituent society of this Society.

Section 2. Application for active or affiliate membership in this Society shall be made on a form prescribed by this Society. The transmittal to the executive secretary of a list of active and affiliate members in good standing in a constituent society, together with their annual dues shall establish them as members of this Society.

Section 3. Application for student membership in this Society shall be made on a form prescribed by this Society. The transmittal of this application to the executive secretary of this Society together with the required amount of dues shall establish him as a student member of this Society.

Section 4. Any member of this Society whose conduct is detrimental to this Society may be expelled by a two-thirds vote of the House of Delegates provided he has been given thirty days notice, signed by a majority of the members of the Board of Directors and sent by registered mail, stating the charges filed against him and informing him of the date of the meeting when his expulsion will be voted upon. The member so charged shall have the privilege of appearing before the House of Delegates and he may present his defense in person or through other active members of the Society of his selection.

Section 5. Any person who has been expelled from membership may be reinstated upon adoption by a two-thirds vote of the House of Delegates of his application for membership, provided the same is accompanied by annual dues to this Society plus a reinstatement fee of five dollars ($5.00).

ARTICLE V

Code of Ethics

Being fully cognizant of my responsibilities in the practice of Medical Technology, I affirm my willingness to discharge my duties with accuracy, thoughtfulness, and care.

Realizing that the knowledge obtained concerning patients in the course of my work must be treated as confidential I hold inviolate the confidence (trust) placed in me by patient and physicians.

Recognizing that my integrity and that of my profession must be pledged to the absolute reliability of my work, I will conduct myself at all times in a manner appropriate to the dignity of my profession.

ARTICLE VI

Meetings

Section 1. The Society shall hold an Annual Session which shall include one or more general or scientific meetings of the members and not less than two meetings of the House of Delegates, at least two of which shall be held on different days. The Annual Session shall be held at the time and place determined by the House of Delegates at any preceding Annual Session. Emergency changes concerning the time and place of an Annual Session may be made by the Board of Directors.

Section 2. The Board of Directors shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within the two weeks prior to the first meeting of the House of Delegates at each Annual Session.

Section 3. The Advisory Council shall meet within the week following the sine die adjournment of the House of Delegates to transact necessary business and shall hold a meeting within two weeks prior to the first meeting of the House of Delegates of each Annual Session.

ARTICLE VII

Finances

Section 1. The dues for active and affiliate membership in this Society shall be twelve dollars ($12.00) per annum.

Section 2. (a) Dues for student membership in this Society shall be $3.00 per annum. (b) Any dues paid by a student member who becomes eligible and applies for active membership prior to January 1 will be refunded on the basis of $.25 per month for any overlapping period retroactive to July 1.

Section 3. The dues for corresponding membership in this society shall be four dollars ($4.00) per annum.

Section 4. (a) Dues to this Society shall be payable to the treasurer of the constituent Society of the geographical area in which the member is employed, resides, or maintains his status as a student, or in the event no such constituent society exists, to the executive secretary of this Society.

(b) Annual dues for active, affiliate and corresponding members to this Society are payable on or before July 1 of each fiscal year.
(c) Dues for a student member may be renewed at the end of 12 months for another year, provided he has not in the interim become eligible for active membership in this Society.

Section 5. (a) Any active, affiliate, or corresponding member who cannot pay dues for the current fiscal year shall notify this Society by sending written notification direct to the executive secretary not later than September 1st of that year.
(b) Any active, affiliate, or corresponding member who fails to pay annual dues to this Society by September 1st shall have his name removed from the membership list.
(c) A person whose name has been removed from the membership list of this Society may be reinstated at any time provided he meets the requirements for membership of both his constituent society and this Society. Unless a person has previously resigned, to be reinstated in this Society he shall pay current annual dues to his constituent society with any back dues or reinstatement fee the constituent society may require, and current year's dues to this Society plus a reinstatement fee of two dollars to this Society.
(d) Any active or affiliate member who changes his residence or place of employment to another state, territory or nation shall become a member of the constituent society of his new residence or place of employment for the remainder of the fiscal year without further payment of dues.

Section 6. A new applicant elected to active, affiliate, or corresponding membership in this Society after January 1st and prior to May 1st shall pay one-half of the annual dues to this Society for the remainder of the fiscal year.

Section 7. An applicant elected to active, affiliate, or corresponding membership in this Society between May 1st and June 30th shall pay annual dues for one year which shall entitle him to all the privileges of his respective membership for a period up to fourteen months. He shall pay no further dues to this Society until the second fiscal year following the date of his application.

ARTICLE VIII

Officers

Section 1. The officers of this Society shall be president, president-elect, recording secretary and treasurer.
(a) The president shall be the chief executive of this Society and shall preside at all meetings of the Board of Directors and of the House of Delegates. He shall be an ex officio member of all standing and special committees except the nominations committee. He shall set up such standing committees as are provided for in the Bylaws except the nominations committee. He shall appoint with the approval of the Board of Directors, such special committees as are needed.
(b) The president-elect shall, during his term of office, familiarize himself with the duties which shall devolve upon him as president. In the event of the death, resignation or total disability of the president, the president-elect shall perform the duties of, and have the same authority as, the president.
(c) (1) The recording secretary shall act as secretary at all meetings of the Board of Directors and of the House of Delegates.

(2) He shall keep in permanent form in his possession a record of all minutes taken at all meetings at which he serves, and shall provide each of the members of the Board of Directors and the executive secretary with a copy of these minutes within thirty days after the close of the Annual Session at which the minutes were taken.

(3) He shall, assisted by the minutes committee, prepare an abstract of the minutes to be published and distributed to the membership.

(4) He shall carry on such correspondence as shall be authorized by the House of Delegates and Board of Directors of this Society.

(5) To aid the recording secretary in the keeping of an accurate record of the annual meetings of the House of Delegates the Society shall employ

a mechanical recording device or an expert stenographer to record all the proceedings of the meetings of the House of Delegates. Such record shall be delivered to the president of this Society by the stenographer within sixty days after the close of an Annual Session. The president shall within sixty days after receipt of the record transmit the same to the executive secretary to be filed in the executive office.

(6) In the event of the simultaneous inability of both the president and the president-elect to perform the duties of president, the recording secretary shall perform the duties of, and have the same authority as, the president.

(d) The treasurer shall receive, record and have charge of all funds of this Society. He shall deposit these funds in a bank designated by the Board of Directors and pay all bills approved by the Board of Directors or the House of Delegates upon receipt of vouchers signed by the chairman of the Board. He shall be bonded at the expense of the Society. He shall, within sixty days previous to the Annual Session, have his accounts audited by a public accountant at the expense of the Society. He shall submit to the House of Delegates at each Annual Session an itemized statement of all receipts and expenditures of the Society for the year just ended, and a copy of this itemized statement shall be distributed to each delegate prior to the reading of the report. At the end of his term of office he shall have a final interim audit of his accounts and shall deliver his current records and all cash on hand to his successor. All other records in his possession shall be transmitted to the executive secretary to be filed in the executive office.

(e) At the end of the term of office by expiration or otherwise each officer, except the treasurer, each Board member and Council member, shall deliver his official records and correspondence to his successor for transmittal to the executive secretary to be filed in the executive office.

Section 2. Eligibility for Office. Any person who is, and for at least three years prior to his election or appointment has been, an active member of this Society shall be eligible to hold office.

Section 3. Method of Election.

(a) All officers of this Society shall be elected by the House of Delegates at the Annual Session of this Society.

(b) An official list of nominees for each office shall be presented to the House of Delegates by the nominations committee, the list of candidates and their qualifications having been sent to each member of this Society at least sixty days before the Annual Session. Nominations may be made from the floor. The qualifications of all persons nominated from the floor shall be presented to the House of Delegates at the time such nominations are made.

(c) The election of officers by the House of Delegates shall be conducted by an elections committee consisting of five members of the House of Delegates appointed by the chair.

(d) Election shall be by ballot and a majority of votes cast elects. If none of the candidates for a certain office receives a majority vote on the first ballot, the candidate with the lowest number shall be eliminated and balloting shall continue until a candidate receives a majority of the votes cast. In the event of a tie, election shall be determined by lot.

Section 4. Term of Office.

(a) The president-elect shall be elected for a term of one year and, except in the event of a vacancy in the office of president by death, resignation or total disability, he shall serve for one year as president-elect and then automatically succeed to the office of president.

(b) The recording secretary shall be elected for a term of one year. He may be reelected. He shall not serve in this office for more than two years.

(c) The treasurer shall be elected for a term of three years. He may be reelected. He shall not serve in this office for more than six years.

(d) The term of office of any officer of this Society elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 5. Filling of Vacancies.

(a) In the event of the death, resignation or total disability of the president, the president-elect shall become the president of this Society for the remainder of the year of the president's term of office and for the following year, and the Board of Directors shall elect by majority vote a president-elect to serve for the remainder of the year in which the vacancy occurs. At the next Annual Session following the occurrence of the vacancy in the office of president, the House of Delegates shall elect a president-elect for a term of one year.

(b) In the event of the simultaneous removal during their term of office of both the president and the president-elect by death, resignation or total disability, the recording secretary shall become the president of this Society for the remainder of their term of office. The Board of Directors shall by majority vote elect a president-elect from the slate of nominees for that office from the previous election and a recording secretary from the slate of nominees for that office from the previous election, both of whom shall be elected to serve for the remainder of the year in which the vacancies occur.

(c) In the event of removal from office of the recording secretary by death, resignation or total disability, the Board of Directors shall by majority vote elect a recording secretary from the slate of nominees for that office from the previous election to serve for the remainder of the year in which the vacancy occurs.

(d) In the event of removal from office of the treasurer by death, resignation or total disability, the Board of Directors shall by majority vote elect a treasurer from the slate of nominees for that office from the previous election to serve for the remainder of the term of office for which his predecessor was elected.

ARTICLE IX
Executive Secretary

Section 1. The executive secretary shall be elected by the Board of Directors, shall serve under a written contract, and, subject to the action of the House of Delegates, shall be under the supervision and control of the Board. He shall, under the direction and control of the House of Delegates and the Board of Directors, maintain a central office for the Society in which the records, properties, bonds and legal papers of the Society shall be preserved in properly maintained files. He shall do the administrative work necessary to the operation of the Society and shall be the business manager of the official publication.

Section 2. The executive secretary shall contract for technical exhibits for the Annual Session of the Society and, working with the local chairman of exhibits, shall arrange for all technical exhibits. All receipts from technical exhibits shall go into the treasury of this Society.

Section 3. The executive secretary shall be included as a non-voting member of the Board of Directors and of the Advisory Council.

Section 4. The executive secretary shall distribute to the president, the president-elect and the other officers, and to the committee chairmen and to Advisory Council members, the names and addresses of the officers and committee chairmen of constituent societies as soon after October 1 as their membership status in this Society can be determined, and such lists prepared.

Section 5. An assistant executive secretary, preferably a Medical Technologist (ASCP), shall be appointed by the executive secretary with the approval of the Board of Directors. He shall be salaried, and shall work with and under the direction of the executive secretary so that he may become familiar with all phases of the duties of the executive secretary in the central office of this Society. In the event of the resignation, total disability or death of the executive secretary, the assistant executive secretary shall maintain the continuity of the functions of the central office of this Society. In such event, he shall serve as acting executive secretary for a period not to exceed one year and shall become executive secretary only upon the approval of the Board of Directors.

ARTICLE X
Board of Directors

Section 1. The Board of Directors shall consist of the president, the president-elect, the recording secretary, the treasurer, the immediate past-president and six members of this Society elected by the House of Delegates.

Section 2. The Board of Directors shall represent the Society when the House of Delegates is not in session and only when urgent business necessitates immediate action that cannot wait until the House of Delegates reconvenes. In no case, except in an emergency, shall the Board of Directors transact business that vitally affects the interests of this Society and in no case shall the Board of Directors take any action which binds the Society beyond the next ensuing Annual Session, except as specific authority for such action has been previously granted by the House of Delegates.

Section 3. Any person who is, and for at least three years prior to election or appointment has been, an active member of this Society shall be eligible to serve on the Board of Directors.

Section 4. Method of Election.

(a) Two members of this Society shall be elected annually to the Board of Directors by the House of Delegates each to serve for a term of three years.

(b) The nominations committee shall prepare two slates of nominees, one for each vacancy on the Board. Nominations may be made from the floor. The qualifications of all persons nominated from the floor shall be presented to the House of Delegates at the time such nominations are made.

(c) Election shall be conducted by the elections committee.

(d) Election shall be by ballot, voting continuing until a candidate receives a majority vote.

Section 5. The term of office of any member of the Board of Directors elected at an Annual Session shall begin with the sine die adjournment of the House of Delegates at such Annual Session.

Section 6. The president of this Society shall serve as chairman of the Board of Directors and the recording secretary shall serve as secretary of the Board.

Section 7. The president or president-elect and seven other voting members of the Board shall constitute a quorum.

Section 8. The annual meeting of the Board of Directors shall be held within two weeks prior to the first meeting of the House of Delegates at each Annual Session, the time and place of such meeting being designated by the chairman.

Section 9. In the event of removal by death, resignation or total disability of a member of the Board of Directors who is not an officer of this Society, the president of this Society shall appoint one of the candidates from the slate of nominees for members of the Board of Directors from the preceding election to serve until the next Annual Session. At the next Annual Session the vacancy shall be filled by election by the House of Delegates.

Section 10. The method of carrying on the business of the Society between the regular annual meetings of the Board of Directors shall be:

(a) The material shall be submitted in writing in two copies, one to the president, the second to the executive office for filing. Full reasons for the proposed action shall be stated, and the proposal shall be accompanied by the second of another member of the Board.

(b) Within fifteen days of receipt of material, the president shall submit the proposal, accompanied by the reasons, to the executive office with instructions that it be sent to the Board of Directors for their approval and returned to the executive office.

(c) The results of the vote of the Board of Directors shall then be sent to each Board Member by the executive office.

ARTICLE XI
House of Delegates

Section 1. The membership of the House of Delegates shall consist of the members of the Board of Directors, the members of the Advisory Council and the delegates elected by their constituent societies or appointed in accordance with the provisions of these Bylaws.

Section 2. Each constituent society shall hold an annual election for the purpose of the election of delegates to represent the members of said constituent society at annual or special sessions of the Society.

Section 3. (a) The basis of representation is as follows: One delegate and one alternate for each 25 active members, or major fraction thereof of the constituent society. When the membership of a constituent society exceeds 250 active members, the basis of representation shall be one delegate and one alternate for each 50 active members, or major fraction thereof, above 250.

(b) The number of delegates to which each constituent society is entitled shall be determined by the number of active members who have paid current dues to and are in good standing with this Society. A member who has transferred from one constituent society to another shall be counted as a member of the geographical area in which he holds his membership thirty days prior to the opening of the Annual Session.

(c) Notwithstanding anything to the contrary, each constituent society shall be entitled to at least one delegate, and no delegate or delegates representing a constituent society shall have a greater number of votes than the total membership of said constituent society.

Section 4. The secretary of each constituent society shall send to the executive secretary of this Society at least two weeks before the opening of the Annual Session a list of delegates and alternates from his society with their addresses and registry numbers or academic degrees.

Section 5. If the combined number of delegates and alternates present at any Annual Session from any one constituent society is less than the quota of delegates from that society, the president of that constituent society, or in his absence the other officers of that society, shall fill the quota from eligible active members of the constituent society present at the Annual Session. When necessary the president of this Society shall name these delegates.

Section 6. Each delegate and each alternate shall present his credentials to the chairman of the convention credentials committee when he registers at the Annual Session.

Section 7. The members of the Board of Directors and of the Advisory Council and the officers of this Society shall not be included in the list of elected or appointed delegates from a constituent society to the House of Delegates.

Section 8. Delegates and alternates shall serve from the time of the first meeting of the House of Delegates at the Annual Session for which they were elected as delegates and alternates to the first meeting of the House of Delegates at the next ensuing Annual Session.

Section 9. A quorum of the House of Delegates shall be a majority of its accredited members registered at the Annual Session.

Section 10. Any member in good standing in this Society is eligible to attend the annual meetings of the House of Delegates and to participate in discussion but the privilege of the floor shall be granted to a non-delegate only by consent of the House. Only members of the House of Delegates may vote.

ARTICLE XII

Advisory Council

Section 1. (a) The Advisory Council of this Society shall consist of those persons who are the presidents and presidents-elect of the constituent societies or, in the absence of a president-elect such other person as may be selected by the constituent society, and those persons who are the members of the Board of Directors of this Society as of the day immediately following the sine die adjournment of the House of Delegates at any Annual Session. These same persons shall remain on the Advisory Council until the sine die adjournment of the House of Delegates at the next ensuing Annual Session even though in that period the presidents of constituent societies on the Council have become the past presidents and the presidents-elect have become presidents of their respective constituent societies.

(b) With the exception of the members of the Board of Directors, it shall

be the privilege of a member of the Council to appoint any other active member of his constituent society as his alternate when he is unable to be present at a meeting of the Council, provided that only two active members from a constituent society shall be allowed certification as members of the Advisory Council at any one Annual Session of this Society.

Section 2. The purpose of the Advisory Council shall be to consider matters of importance pertaining to the interest and welfare of the members of this Society and of the members of the profession of medical technology in general. The Advisory Council shall have no power to act for the Society. It may make recommendations to the House of Delegates. By means of a report made by the chairman or other duly authorized member of the Council to the House of Delegates it may bring matters of importance to the attention of the House of Delegates.

Section 3. Officers of the Advisory Council shall consist of a chairman, a vice-chairman, a recording secretary, an assistant recording secretary and a corresponding secretary.

(a) Officers of the Advisory Council shall be elected at a meeting following soon after the sine die adjournment of the House of Delegates at each Annual Session.

(b) No member of the Board of Directors shall hold office in the Advisory Council.

(c) The retiring chairman of the Advisory Council shall call a meeting of the Advisory Council soon after the sine die adjournment of the House of Delegates for the purpose of organizing the Advisory Council. He shall act as chairman pro tem of this meeting. He shall read, or distribute in writing, to the members of the Council a list of the persons eligible for office. Nominations shall be made from the floor. Election shall follow. A majority vote of all members of the Advisory Council present at that meeting of the Council shall elect.

(d) A quorum of the Advisory Council shall be a majority of its members.

(e) Officers shall take office immediately after their election and they shall remain in office until the sine die adjournment of the House of Delegates at the next ensuing Annual Session.

Section 4. The duties of the officers of the Advisory Council shall be as follows:

(a) The chairman shall preside at all meetings of the Council. He shall provide for the preparation and presentation of reports and recommendations from the Advisory Council to the House of Delegates.

(b) The vice-chairman shall in the absence of the chairman assume the duties of the chairman.

(c) The recording secretary shall take the minutes of the meetings of the Advisory Council and shall keep a permanent record of these minutes. He shall, within thirty days after the close of the Annual Session, prepare and distribute to the members of the Advisory Council a copy of the minutes of the meetings held by the Council at the time of the Annual Session just closed.

(d) The assistant recording secretary shall in the absence of the recording secretary assume the duties of the recording secretary.

(e) The corresponding secretary shall carry on the correspondence for the Advisory Council.

Section 5. Any member in good standing in this Society shall be eligible to attend the meetings of the Advisory Council and to participate in discussion, but the privilege of the floor shall be granted to a non-member of the Council only by consent of the Council. Only members of the Council may vote.

(a) Each constituent society shall be entitled to two and only two voting members at any meeting of the Council.

Section 6. The routine operating expenses of the Advisory Council shall be included in the annual budget of this Society. Any request for an appropriation for special activities of the Council shall be presented to the Board of Directors for approval.

ARTICLE XIII
Representation on the Board of Schools

Section 1. The president, the president-elect and the immediate past president of this Society shall represent the Society as members of the Board of Registry of Medical Technologists of the American Society of Clinical Pathologists.

ARTICLE XIV
Representation on the Board of Schools

Section 1. Three representatives of this Society shall be elected by the House of Delegates to serve on the Board of Schools of the American Society of Clinical Pathologists. Nominations shall be made by the nominations committee and election shall be by ballot. A majority of the votes cast elects.

Section 2. Persons who are, and for at least five consecutive years immediately prior to election or appointment have been, active members in good standing of this Society shall be eligible for election to the Board of Schools provided, however, that such persons shall hold academic degrees at least at a baccalaureate level and have had at least three years of teaching experience within the current decade in a school of medical technology approved by the Council on Medical Education and Hospitals of the American Medical Association and that they shall have held office either in this Society or in one of its constituent societies or have held membership on the Board of Directors of this Society or on a comparable Board of a constituent society.

Section 3. A representative to the Board of Schools shall be elected to this position for a term of three years. One representative shall be elected at each Annual Session.

Section 4. A representative to this Board shall not serve for more than two terms or more than six years.

Section 5. In the event of the death, resignation or total disability of a representative of this Society to the Board of Schools, the Board of Directors shall fill the unexpired term by appointment.

ARTICLE XV
Elections Committee

All elections by the House of Delegates shall be conducted by an Elections Committee consisting of five delegates appointed by the chair. Each member of the elections committee shall receive and carry out the list of instructions previously prepared by the chair in accordance with the parliamentary authority of the Society.

ARTICLE XVI
Special Committees

Section 1. There shall be a nominations committee composed of seven active members of this Society, no two of whom shall be from the same constituent society and none of whom shall be an officer of this Society.

(a) The House of Delegates shall elect the nominations committee. Nominations for membership on this committee shall be made only from the floor of the House. The time for these nominations shall be fixed for the first day of the meetings of the House of Delegates at each Annual Session, and each constituent society shall be entitled to make only one nomination. The time for election of the nominations committee shall be fixed for the second day of the meetings of the House of Delegates at each Annual Session.

(b) Election shall be by ballot, and each delegate shall be provided with a printed ballot bearing names of all nominees. The seven persons receiving the highest number of votes shall be considered elected. The person receiving the highest number of votes shall serve as chairman of the committee.

(c) In the event of a vacancy due to death, resignation or refusal to serve, the Board of Directors shall elect by majority vote an eligible member of this Society to fill the vacancy. The remaining nominees for this committee shall be considered first, in the order of the highest number of votes received.

(d) The nominations committee shall provide for the publication in the September-October issue of the official publication of this Society the names

and offices of those officers, directors, and representatives for the Board of Approved Schools whose term of office expires at the next Annual Session.
(e) It shall solicit from the constituent societies or from any active member of this Society, names and qualifications of qualified candidates for such offices and positions, with instructions that such material be in the hands of the nominations committee not later than December 1st. Only the names of members who have consented to serve if elected shall be submitted by a constituent society or by a member of this Society to the nominations committee.
(f) The nominations committee shall prepare a slate of at least two nominees for each office and other position to be filled by the House of Delegates. Not less than sixty days before the date of the next Annual Session, a list of nominees together with their qualifications shall be distributed to each active member of this Society.

Section 2. Other special committees may be appointed at any time by the president on authority given him by the House of Delegates or the Board of Directors.

Section 3. The president shall appoint the necessary personnel for committees that shall arrange for the Annual Session of this Society.

Section 4. The President shall appoint a minutes committee with authority to approve and correct the minutes of the previous Annual Session of the House of Delegates. An abstract of these minutes and of the minutes of the meetings of the Board of Directors held since the first meeting of the House of Delegates of the last Annual Session shall be published and distributed to members not less than 60 days prior to the next Annual Session.

ARTICLE XVII
Standing Committees

Section 1. There shall be the following nine standing committees: Constitution and Bylaws; Education; Legislation; Membership; Public Relations; Research; Service Fund and Finance; Standards and Studies; Recruitment. Each of such committees shall be composed of six members, with such geographical distribution as shall be conducive to effective work.

Section 2. At the beginning of his term of office the president of this Society shall appoint, each for a term of three years, two members to each of the standing committees to succeed the two members whose term ended with the sine die adjournment of the House of Delegates at the Annual Session. The president shall appoint a chairman for each standing committee for a term of one year. A member may not serve for more than six consecutive years on the same standing committee. Only active members may be appointed to membership on standing committees.

Section 3. The duties of the standing committees shall be as follows:
(a) The Constitution and Bylaws Committee shall:

(1) Examine all proposed amendments to the Bylaws of this Society. Proposed amendments to the Bylaws of this Society may be submitted by an active member of this Society or by a constituent society. After the committee makes such changes as are necessary to put these proposed amendments in proper form without altering their intent, it shall have them distributed to each active member of this Society not less than sixty days prior to the next ensuing Annual Session of this Society.

(2) Thirty days after the adoption of an amendment or amendments to the Articles of Incorporation or Bylaws of this Society, the recording secretary shall send an official report of such adoption to the executive secretary who shall provide for publication of the announcement of adoption of such amendments in the September-October issue of the official publication of this Society.

(3) The Constitution and Bylaws Committee shall examine the proposed constitution, or Articles of Incorporation, and bylaws of any group petitioning for a charter from this Society and shall bring a report and a recommendation concerning the chartering of such group to the House of Delegates at the next ensuing Annual Session of this Society.

(4) The Constitution and Bylaws Committee shall examine all proposed amendments to the constitution and bylaws of a constituent society.

It shall submit the same to the chairman of the Board of Directors with an opinion only if a majority of the committee members disapprove the proposed amendments. In such case these must be approved by a majority of the Board of Directors before the proposed amendments are presented to the membership of the constituent society to be voted on in the manner prescribed by the bylaws of said society.

(b) The Education Committee shall develop teaching aids, encourage the establishment of laboratory libraries, prepare and make available to members and to constituent societies current lists of visual aids, study the curricula of approved schools of medical technology, make suggestions and receive such from the Board of Schools, and aid graduate and undergraduate students of approved schools of medical technology in every way possible.

(c) The Legislation Committee shall assume the duties assigned to it by the House of Delegates and Board of Directors.

(d) The Membership Committee shall plan ways and means of cooperating with the membership committees of constituent societies with the aim of increasing the membership of this Society and of the constituent societies.

(e) The Public Relations Committee shall, by dispensing information and promoting public understanding of the profession of medical technology, act as a liaison group between this Society and other professional or lay groups.

(f) The Research Committee shall direct the research activities of this Society.

(h) The Service Fund and Finance Committee shall stimulate fund raising for service to the undergraduate and graduate medical technologist and shall comply in so far as possible with the suggestions of the Board of Directors and House of Delegates regarding policies and practices relative to the use of the fund. Disbursements from the fund shall be made by voucher signed by the chairman of the Board of Directors. A separate financial account shall be kept for the fund. This shall be audited as provided for the other financial accounts of the Society. At each Annual Session of this Society an itemized statement of all receipts and expenditures to the fund during the past year shall be presented to the House of Delegates. The Service Fund and Finance Committee shall also study ways and means of meeting allotments established in the budget and shall prepare a budget for the coming year to be presented to the House of Delegates at each Annual Session.

(i) The Standards and Studies Committee shall study medical technology ethics, economics, standards and other kindred problems. This committee shall inform the members of this Society of the activities of other professional medical technology organizations. It shall promote better understanding of professional medical technologists by the public so that the public may discriminate between adequately and inadequately trained members of our profession.

(j) The Recruitment Committee shall have the responsibility of recruiting students into the field of medical technology.

ARTICLE XVIII
Fiscal Year

The fiscal year shall be from July 1 to June 30, inclusive.

ARTICLE XIX
Official Publication

Section 1. The official publication of this Society shall be THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, the name of which is patented and the contents of which shall be copyrighted.

Section 2. The official publication shall be made available to all active, affiliate, corresponding and student members of this Society who have paid their dues and are in good standing, and to all honorary members.

Section 3. The editor of the official publication shall be elected by the Board of Directors and shall serve under written contract.

Section 4. The Editorial Board shall consist of the editor, two or more advisory editors and two or more associate editors. The advisory and associate editors shall be appointed annually by the editor with the approval of the Board of Directors.

Section 5. All editorials shall be approved by the editor-in-chief and one other member of the Editorial Board before publication.

Section 6. All papers read at convention or submitted to the Society shall become the property of the Society and shall be published, if warranted, in THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY under the copyright of the society unless a petition for the privilege of prior publication has been obtained of the Editorial Board.

ARTICLE XX

The excutive office of the Society shall be located at Houston, Texas.

ARTICLE XXI

Parliamentary Authority

Robert's Rules of Order Revised shall govern the business proceedings of this Society except when otherwise specified in these Bylaws.

ARTICLE XXII

Amendments

Section 1. The Bylaws of this Society may be amended as follows:

(a) Any proposed amendment to these Bylaws recommended by either a constituent society or by an active member of this Society shall be submitted in writing to the chairman of the Constitution and Bylaws Committee not later than December 1st. Twelve copies of the proposed amendment in context shall be submitted. The Constitution and Bylaws Committee shall make such changes as may be necessary to provide proper form without altering the meaning of the amendment. After such changes have been made the proposed amendment shall be distributed to each active member of this Society not less than sixty (60) days prior to the opening of the next Annual Session, at which Session the proposed amendment shall be presented to the House of Delegates for vote.

(b) Amendments to these Bylaws shall be adopted by a two-thirds vote of the House of Delegates, except that a proposed amendment to Article XX of these Bylaws shall be approved by a two-thirds vote of the Board of Directors before it may be submitted to the House of Delegates for vote.

Section 2. A complete revision of these Bylaws shall be presented to a meeting of the House of Delegates at an Annual Session only upon two-thirds vote of the House of Delegates at one Annual Session ordering a revision of the Bylaws for presentation to a meeting of the House of Delegates at the next ensuing Annual Session. A two-thirds vote of the House of Delegates shall be necessary for adoption of a revision of these Bylaws.

Section 3. Manner of voting on amendments to Articles of Incorporation:

(a) Amendments shall be made only at an Annual Session of the House of Delegates by a majority vote of the total number of its accredited members. The vote shall be by roll call.

(b) In the event that the authorized quota of accredited delegates of any constituent society is unable to be present at the Annual Session of the House of Delegates, such constituent society shall be entitled to send the duly certified votes of its absent accredited delegates to the executive secretary 10 days before the date of said Annual Session, such votes to be cast at the time the roll call vote is taken.

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TO CONTRIBUTORS AND CORRESPONDENTS OF THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY

THE AMERICAN JOURNAL OF MEDICAL TECHNOLOGY is devoted to the publication of original investigation and observations in medical technology. Manuscripts, books for review, and inquiries concerning material to be published, or advertising should be forwarded to:

Miss Rose Matthaei, MT (ASCP)—Editor
The American Journal of Medical Technology
Suite 25, Hermann Professional Building
Houston 25, Texas

MANUSCRIPTS: Manuscripts must be submitted in original typewritten or otherwise duplicated copy, with all written matter double or triple spaced. Separate sheets should be used for each table, for references, and for legends. References should include only articles cited in the text and must be arranged in alphabetic sequence and numbered in that order, each reference being arranged as follows: surname and initials of author, title of article in lower case except where a capital letter is required, abbreviation of name of periodical (see Quarterly Cumulative Index Medicus), volume in Arabic numerals, inclusive pages, year. References to books should include total pages or, if indicated, pages to which reference is made. References made in the text itself must be by name and date, i.e. "developed blood agar (Haley, 1951)."

Nomenclature for micro-organisms should be based on Bergey's manual. Standard abbreviations, only, should be used in the text, and one should refrain from an excess of footnotes. Manuscripts are accepted for publication on the condition that they are contributed exclusively to the AMERICAN JOURNAL OF MEDICAL TECHNOLOGY, or that they have previously been presented before a convention of the American Society of Medical Technologists. (They may also have been published in a constituent society newsletter or journal or presented before a constituent society convention, provided this information is included in an explanatory letter to this journal.)

Manuscripts submitted to the journal for consideration for publication must be in final form. No rewriting, no changes with regard to illustrations, etc., will be allowed. In certain cases, it may be acceptable to add a very short appendum. In general, the format of the paper should be outlined as follows:

1. Introduction	3. Results	5. Summary
2. Methods	4. Discussion	6. References

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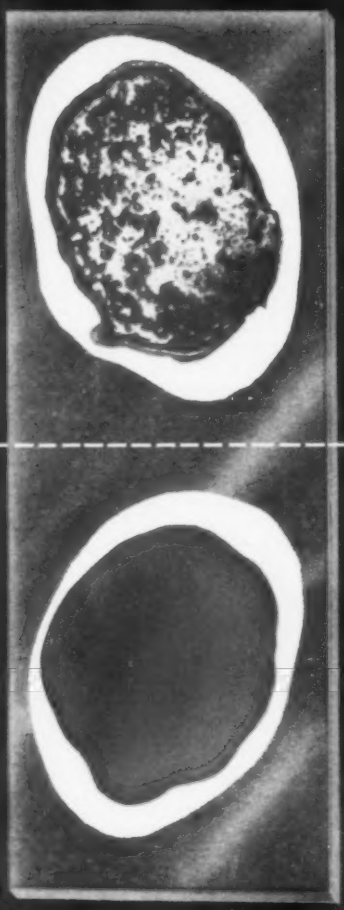

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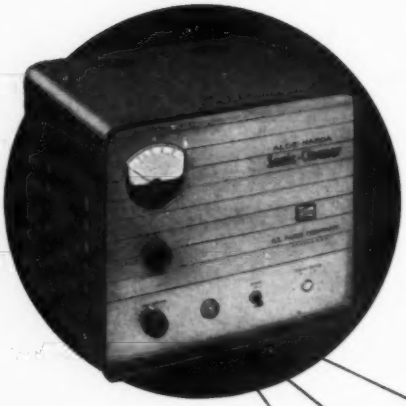

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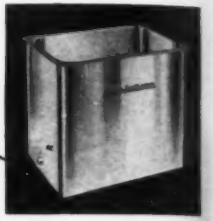

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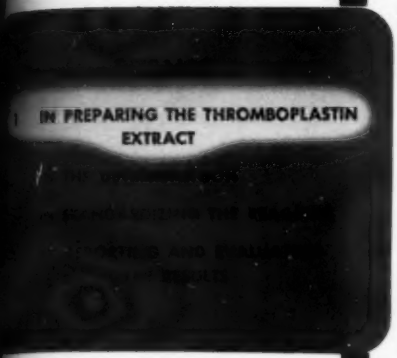

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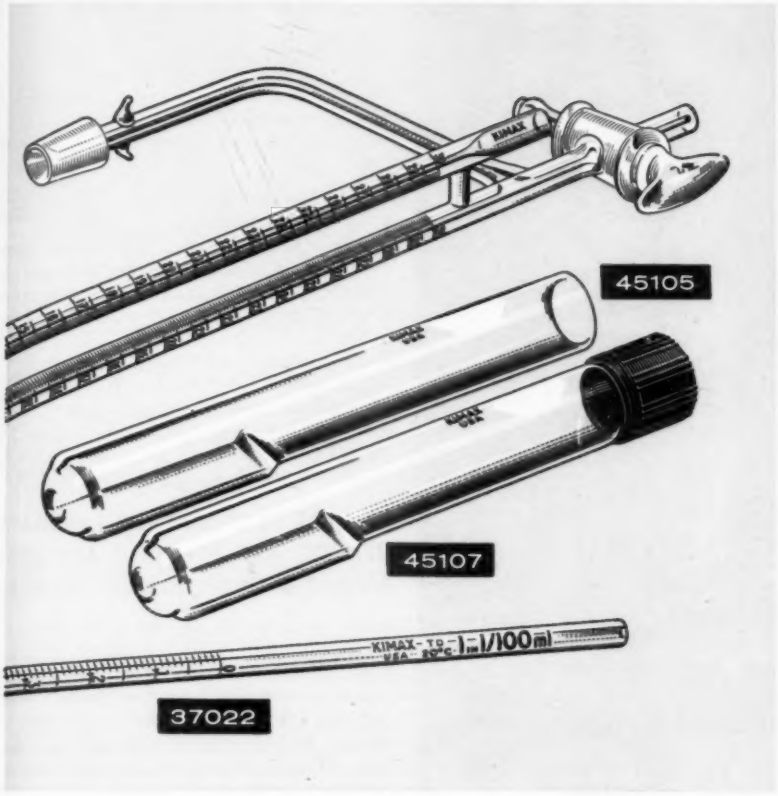

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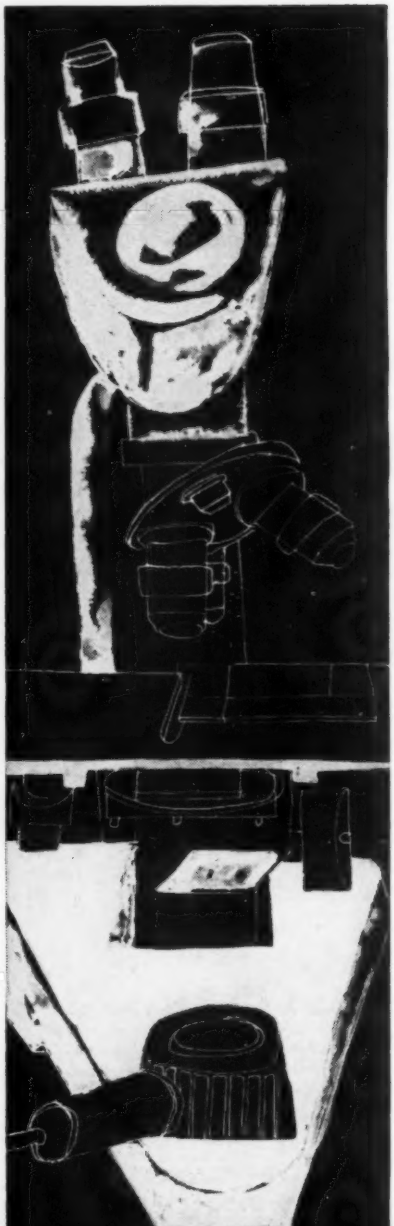

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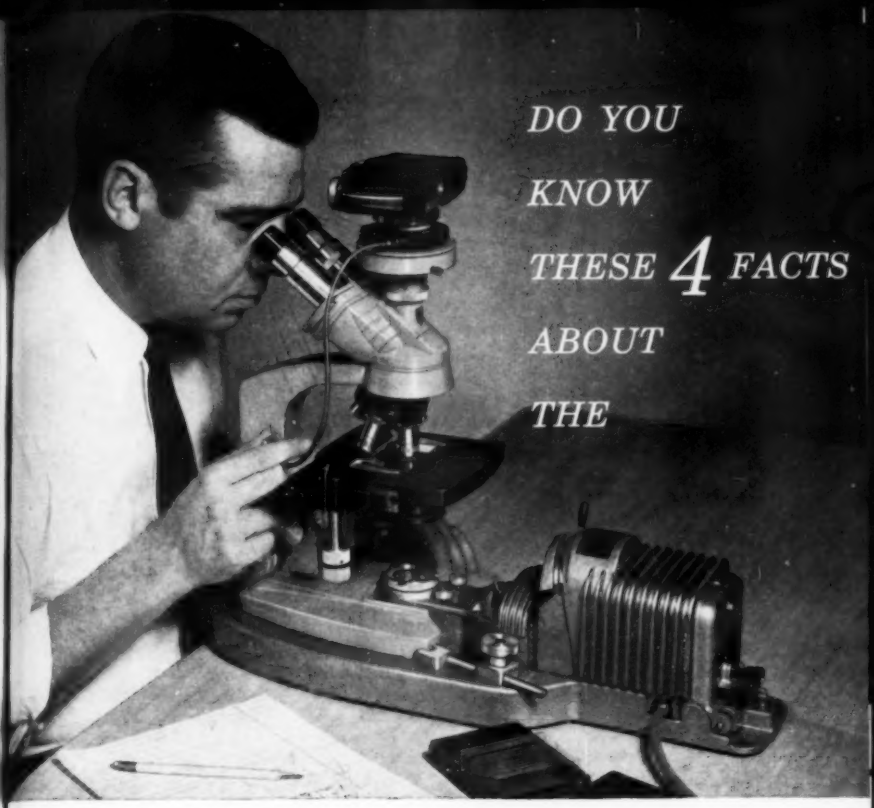

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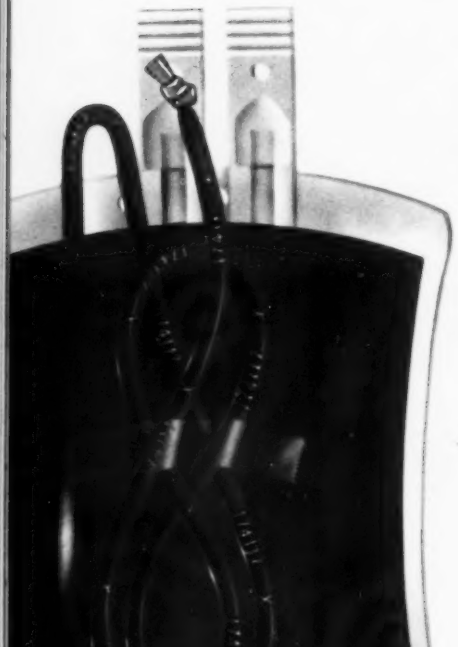
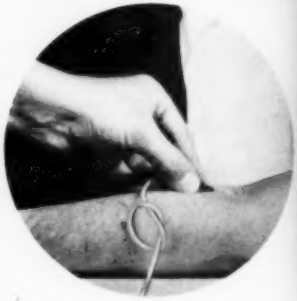


1 Prepare site for phlebotomy



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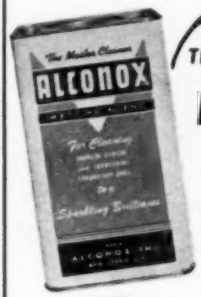

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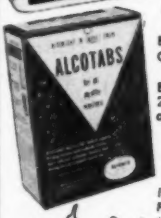

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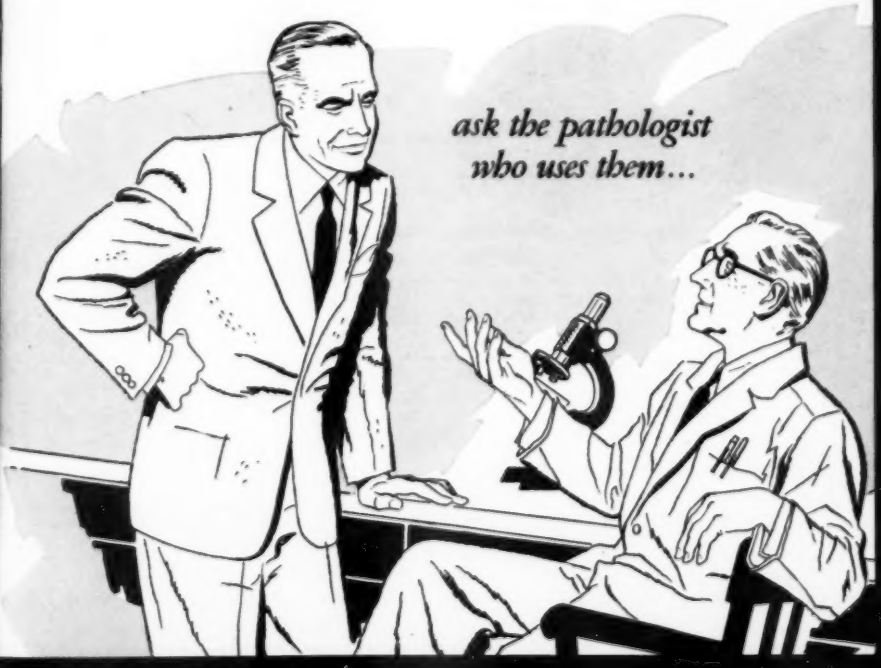

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